Medical Research Council

Institute for Environment and Health

IEH report on

NATURAL AND MAN-MADE MINERAL FIBRES: UK RESEARCH PRIORITIES

A REPORT COMMISSIONED BY THE MRC COMMITTEE ON TOXIC HAZARDS IN THE ENVIRONMENT AND WORKPLACE

THIS PUBLICATION IS BASED ON WORKSHOPS HELD IN LEICESTER

14 OCTOBER 1994 and 11 JANUARY1995

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Preface

The Medical Research Council's Committee on Toxic Hazards in the Environment and Workplace (MRC CTHEW) advises the MRC on issues related to the identification of toxic effects of agents, the understanding of mechanisms of toxicity, and the reduction of adverse effects in humans arising from exposure to toxic agents. The Committee's remit is to review research in the field of occupational and environmental toxic hazards and to advise the MRC Physiological Medicine and Infections Board on priority areas for research.

This report on research needs in the field of mineral fibres has been prepared at the request of MRC CTHEW. The report is based on two workshops, chaired by Dr I Purchase and organised by the Institute for Environment and Health in October 1994 and January 1995. Participants at the workshops were from major research funding organisations, major research groups, and from industry. The extensive appendix to the report is a series of review papers prepared by workshop participants; these reviews were used as background papers during the workshops.

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LEICESTER, UK, 14 OCTOBER 1994 AND 11 JANUARY 1995

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Executive summary

The recognition that asbestos causes mesothelioma, asbestosis and bronchial carcinoma in humans has lead to a reduction in its use and a concomitant rise in the development and use of man-made vitreous fibres. Nevertheless, there is currently concern about an apparent age-specific increase in the incidence of mesothelioma in men in the UK, which does not appear to be associated with employment in what had previously been regarded as asbestos using occupations. Recent mesothelioma rates among men born in the 1940s are higher than in any previous generation, illustrating the difficulty of accurate assessment of risk and its control.

Currently available data from epidemiological studies and experimental studies, *in vivo* and *in vitro,* have limited value for the prediction of adverse health effects arising from exposure to existing or novel natural or man-made mineral fibres. There is therefore a need to advance understanding of how and why certain mineral fibres cause adverse health effects, in order to facilitate decisions concerning risk assessment.

An important goal, which would facilitate both applied and basic research, *in vitro* and *in vivo*, is to establish a bank of well characterised standard reference fibres.

Applied research should concentrate in the short-term on the improvement of risk assessments both for existing mineral fibres, which are in use now, and for fibres that are currently under development. Longer-term basic research on the mechanisms of fibre toxicity and carcinogenesis in experimental systems and from epidemiological studies will benefit not only future risk assessments, but also the understanding of the biology of cancer.

A multidisciplinary approach will be needed to resolve the outstanding questions on fibre toxicity. Collaborative studies between research groups and between academia and industry and co-operation between funding agencies to support a national programme of research are therefore encouraged.

Workshop Report

1 Introduction

1.1 THE EXTENT OF THE PROBLEM

The industrial exploitation of asbestos began about one hundred years ago and probably peaked in the 1960s. During the early use of asbestos little attention was paid to workplace hygiene and workers were often exposed to massive levels of airborne fibres. This caused serious, and often fatal, pulmonary interstitial fibrosis; the first formal report of a case was recorded as early as 1899. The first reports of lung cancer in asbestos exposed workers appeared in the 1930s (see Lee & Selikoff, 1979). The link with mesothelioma, an otherwise rare tumour of the pleura or peritoneum, was made in the 1960s (Wagner *et al.,* 1960). Some studies have linked asbestos exposure with tumours at other sites but the evidence is inconclusive.

Increasing concern about these hazards has caused a reduction in the use of asbestos, at least in the developed world. Accompanying this concern has been the growth of an asbestos removal (abatement) industry and the development of alternative materials.

However, the age-specific incidence of mesothelioma in men in the UK, far from falling in parallel with the decrease in asbestos use, is actually increasing. Recent mesothelioma rates in men born in the mid 1940s are higher than in any previous generation. The rates for younger men are lower, but still well above the assumed 'natural rate' for this tumour, suggesting a continuing risk at least for men born in the 1950s. There are as yet no meaningful data for births after the 1950s (Peto *et al.,* 1995). Furthermore, the occupations of some current cases appear to lack any association with what have been regarded as asbestos-using trades. These

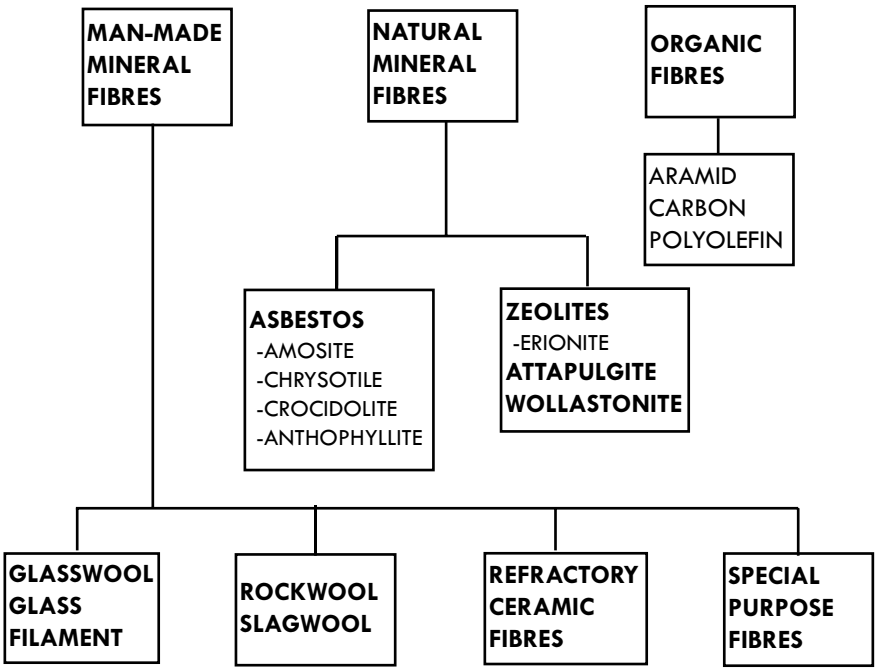
mesotheliomas probably reflect previously unrecognised exposures to asbestos occurring in the building trades perhaps compounded by an underestimate of the potency of certain types of asbestos.

In the UK there are currently approximately 1000 mesothelioma deaths each year. Taking account of the 30-40 year latency period for mesothelioma, it is projected that deaths might reach approximately 3000 per year in 25 years time (Peto *et al.*, 1995). If this is the case, mesothelioma will have changed from an extremely rare to a relatively common condition in the space of 50 years. Additionally, several hundred deaths each year are still ascribed to asbestosis. A similar number of deaths from lung cancer may be due to asbestos exposure, though the synergistic interaction between cigarette smoking and asbestos exposure could mean that this figure is much higher. The importance of this rate of asbestos related deaths can be illustrated by comparison with the much lower death rate from industrial accidents; there were, for example, 379 fatal injuries in UK industry reported in 1993/4 (Health and Safety Commission, 1994). Asbestos is still ubiquitous in buildings; however, as most is still in good condition and covered with decorative coating, exposure should be minimal. Nonetheless, it is important to confirm that substantial exposure is not still occurring. The exposure of those living or working in buildings containing asbestos is generally extremely low, but those involved in the building, removal or abatement industries may still be at risk.

The asbestos minerals are, however, not the only fibrous silicates with industrial and domestic uses. Other mineral fibres, mainly synthetic vitreous fibres made from glass or rock, are used in a variety of ways, most commonly as thermal or acoustic insulation 'wools'. The 'energy crises' of the 1970s triggered an increasing interest in insulation and a rapid growth in the exploitation of these materials. The principal categories of fibres as currently used are outlined in Figure 1 and a general description of the major man-made fibres is provided in Figure 2. In terms of their insulation properties these fibres are not readily replaced by non-fibrous materials, furthermore they have distinct material advantages (toughness, flexibility, wear resistance) relative to potential substitutes. Fibres are also ubiquitous in nature, for example, erionite, another naturally occurring fibrous silicate, also causes mesothelioma. However, in most areas in which they are encountered fibres are innocuous or beneficial.

The association of disease with exposure to asbestos was first detected by clinical observations and epidemiological studies of asbestos workers and confirmed by animal experimentation. Both asbestos and erionite fibres are fine and crystalline, and it is these properties that have been associated with the ability of these fibres

FIGURE 1: MINERAL FIBRES



to cause human disease. There is no clear clinical or epidemiological evidence that synthetic vitreous fibres cause similar diseases to those caused by asbestos in exposed humans but some samples of these fibres cause cancer in experimental animals and in some cases a limited degree of fibrosis (see e.g. IARC, 1988). Many fibrous materials are thought to pose little or no risk as they are too coarse to gain access to the lower respiratory tract, or do not liberate fibrous dust during normal handling and use, or are highly soluble and therefore do not persist in the lung. However with advances in material science, a wide range of fibrous materials, including some organic materials, is being introduced commercially. Some of these fibres have physical properties that may cause concern about their potential to induce disease. It is therefore important to develop methodology to allow at least a preliminary assessment of their potential impact on human health.

FIGURE 2: THE PRINCIPAL MAN-MADE MINERAL FIBRES
(Synthetic vitreous fibres; Man-made vitreous fibres)

Glass
GLASSWOOL is produced by drawing, centrifuging or blowing molten glass.
GLASS FILAMENT is produced from continuously drawn or extruded molten glass.

Rockwool/Slagwool
ROCKWOOL and SLAGWOOL are produced by drawing, centrifuging or blowing molten rock or slag respectively.

Ceramic fibres
CERAMIC FIBRES comprise a wide range of amorphous or crystalline synthetic mineral fibres, characterised by their refractory properties.
Ceramic fibres are produced by drawing, centrifuging or blowing melts or solutions of the appropriate constituents (e.g. alumina, silica, metal oxides).

Special fibres
SPECIAL FIBRES are produced by a variety of methods depending on use. These are frequently for research purposes, e.g. melt drawn, stretched and laser cut to provide uniform length and diameter fibres for research.
Silicon carbide whiskers are used commercially and are produced by a similar process to sublimation and vapour growth.

The increase in the numbers of mesotheliomas, the increasing use of man-made mineral fibres and the continued search for novel fibres and novel applications, all demonstrate the need for continued research into both the mechanism of action and the epidemiology of fibres. It is important to continue mechanistic research on asbestos related diseases in order to advance the understanding of the relation between fibre characteristics and fibre toxicity in general, and to learn how to extrapolate such information to new materials. The primary objective is to ensure

that the risks to human health which have been shown to be associated with some natural crystalline mineral fibres are avoided during the use of newer fibrous materials. In addition the residual risk from asbestos, including the environmental risk from disposal and from natural deposits, requires further examination.

In terms of controlling health hazards from mineral fibres there are two goals of paramount importance. Firstly, there is an urgent need, using available technologies as far as possible, to establish effective and efficient ways of evaluating the health hazard of both novel and existing fibres. Secondly, research into the mechanisms of fibre carcinogenesis must be encouraged; without a proper understanding of mechanisms it will be difficult to introduce either short-term or long-term tests that can accurately predict fibre toxicity. Furthermore, research on fibre-induced cancers will add significantly to an understanding of the process of carcinogenesis *per se*.

A recent European meeting on the assessment of the toxicity of man-made fibres identified a number of important research needs and proposed international collaborative initiatives directed at (i) improving methods to understand better the mechanisms involved in the interaction of fibres with cells and tissues and (ii) promoting an international comparison of different test systems for assessing fibre toxicity (Bignon *et al.,* 1995).

The aim of this report is to make recommendations concerning priorities for research in the UK on natural and man-made mineral fibres. The expectation is that this will provide the impetus to improve the funding and direction of research in fibre toxicity, with the aim of solving some of the outstanding issues involved in assessing any risk from inhaled fibres. This report should therefore make a significant contribution to the ultimate goal of establishing a national programme of research on fibres, within which studies supported by the several funding bodies currently involved in the field can be co-ordinated in order to give the best value research. One of the consequences of such a research programme would be to provide better information on which to base control measures

The report reviews the field, identifies research needs, opportunities and priorities, and discusses the actions that will be required to encourage further progress.

1.2 THE WORKSHOPS

In order to review current knowledge, identify knowledge gaps and establish research priorities for the UK on natural and man-made mineral fibres the Institute for Environment and Health (IEH) convened two workshops to which national and international experts in fibre chemistry, toxicology and epidemiology were invited.

As a first step, a number of participants prepared reviews of current knowledge on fibre characteristics, toxicity, mechanisms of toxicity and epidemiology. The reviews served as background papers for the first workshop and are included as an appendix to this report. During the first workshop the reviews were presented and gaps in knowledge were identified. The workshop participants then worked in subgroups to identify priorities for research. The subgroups were divided according to discipline as follows:

(i) Fibre characteristics and mechanisms of action

(ii) Studies in experimental animals

(iii) Epidemiology and risk assessment

In discussing research priorities, the subgroups were asked to consider research needs and opportunities, both in the UK and abroad, and the strengths and weaknesses in current UK research programmes. Subgroups also took account of barriers to progress such as the lack of certain expertise and methodologies in the UK (which implied a need for training), the fact that certain expertise and methods, although currently available in the UK, were not co-ordinated appropriately (which suggests that a consortium approach is required), and the need for a resource centre to hold standard fibres and reagents. The priorities identified by each subgroup during the first workshop are described in section 2 'Research Needs', which was further refined when the same groups met during the second workshop to finalise their recommendations.

A further key task of the second workshop was to select the priority areas, which were deemed the most likely to provide the scientific insight to assess both the residual risks from exposure to existing mineral fibres and risks from novel fibres in the future. The high priority areas were discussed and further refined by the whole workshop and the overall conclusions of the workshop are presented in section 3 'Recommendations'.

2 Research needs

Each of the three sections in this chapter summarises the discussions of one of the workshop subgroups described previously. Each section outlines gaps in knowledge and research needs, and outlines opportunities for research.

2.1 FIBRE CHARACTERISTICS AND MECHANISMS OF ACTION

Two areas are of particular importance: the durability of fibres and mechanisms of action.

Durability is considered important because a fibre is more likely to be able to inflict significant long-term damage *in vivo* if it is resistant to breakdown and thus persistent in the lungs. The effective dose of fibre is reduced if deposited fibre dissolves. Furthermore, there may be a minimum residence time during which a fibre must be present in order to exert its effect in lung tissue. Additionally, there is the possibility that significant biological modification of the fibres, which may occur with extended tissue residence, is important in the development of disease.

Secondly, study of the mechanisms of pathogenicity is considered to be important because there appears to be something unusual about the fibrous habit that invokes such a significant and detrimental response *in vivo*. This may be a unique

attribute of certain fibres, or the response may be one of degree. Knowledge of the mechanisms of action is needed to facilitate both the process of development of safer alternatives and the development of focused toxicological assays to improve the assessment of human hazard and risk.

Both of these areas of study should be undertaken in the knowledge that fibre dimension has been identified by international scientific consensus as having an important role in the pathogenesis of fibre-related disease.

2.1.1 DURABILITY OF FIBRES IN TISSUES

GAPS IN KNOWLEDGE AND RESEARCH NEEDS

For an understanding of fibre durability in human tissue it is necessary to have effective assays of relative persistence, underpinned by a sound knowledge of how persistence relates both to the physical and chemical characteristics of fibres and to cellular and biochemical events. It is not yet established that existing *in vitro* assays realistically reflect conditions *in vivo.* The key research questions, in no particular order of priority, are:

In what way does the durability of a fibre relate to its physical and chemical composition? What is the nature of the dissolution process?

How does the *in vivo* biological coating (protein or lipid adherence, deposition, opsonisation, etc.) of a fibre affect its solubility?

How does the deliberate coating of industrial fibres (with resins, binders, etc.) affect their solubility?

How does use of a fibre affect its subsequent solubility (e.g. contamination of fibre surfaces during use, or changes in structural composition of fibres as a consequence of heat, chemical treatment, etc.)?

Is the persistence of fibres affected by differences in:

(i) cell type (e.g. macrophages, epithelial or mesothelial cells)

(ii) tissue site (e.g. lung *versus* peritoneum)

(iii) species (human, rat, hamster, etc.)

(iv) intracellular or extracellular residence?

OPPORTUNITIES FOR RESEARCH AND DEVELOPMENT

The structure, composition and residence of fibres in tissue is a critical determinant of fibre toxicity. *In vitro* methods are required to provide data on the dissolution of fibres, which are relevant to *in vivo* exposure.

In the short-term, preferably within a maximum of two to three years, there is a need to identify and validate a limited number of acceptable, relevant and reasonably reliable *in vitro* assays of fibre dissolution. It is considered likely that such assays could be selected pragmatically using existing experimental systems as a starting point, rather than by developing entirely new test systems. It is considered important that the selection of such assays should be on the basis of consensus amongst the material scientists and biologists from academia, industry and the regulatory community, having regard both to relevance *in vivo* and to compositional range of respirable industrial fibres (which can be of mineralogical or organic origin). Finally, whilst it would obviously be ideal if the validation of the selected assays could involve the use of standard reference fibre samples (proposed below) this is not seen as an absolute prerequisite and the lack of availability of any such samples in the short-term should not be a hindrance to progress, particularly since limited supplies of several well characterised samples of various fibres already exist.

There is as yet no widely accepted assay of fibre durability relevant to the *in vivo* setting. Gaining consensus across the academic, industrial and regulatory constituencies about what constitutes an acceptable *in vitro* assay constitutes a possible barrier to progress.

Another important priority is the need for an understanding of the mechanisms of dissolution and/or other processes of fibre breakdown associated with the *in vivo* elimination of some fibre types. The goal of this work will be to gain a full understanding of the key events at the level of the individual fibre, and it is considered likely that these investigations of relative persistence will involve both *in vitro* and *in vivo* experimental systems.

There is a recognised wealth of knowledge and expertise in the UK in this field, both within the academic and the industrial materials science communities. Recognition is growing of the potential added value to be gained from more fully integrating and harnessing the industrial expertise in the research process. There is also currently an important international interest in fibre durability and its potential pathogenic significance. The UK is the location for several of the world's major industrial fibre manufacturers, with a long and documented history of fibre use. There is, however, insufficient regular dialogue or interchange in the UK between those involved in the assessment of hazard from exposure to fibres. It would be valuable to build upon the existing collaborative structures between the funding agencies, to improve the contact between academia and industry, and to initiate more extensive and more effective interdisciplinary research projects within the academic sector, involving industrial interests.

2.1.2 MECHANISMS OF FIBRE TOXICITY

The fundamental questions are considered to relate to mechanisms of the neoplastic response to fibres. The chronic inflammatory sequelae are also important, both in their own right and insofar as they have a role in the modulation or amplification of the carcinogenic process. An holistic view therefore must be taken of the cellular and molecular events associated with fibre pathogenicity.

GAPS IN KNOWLEDGE AND RESEARCH NEEDS

Characterisation of the biochemical and cellular events associated with exposure to fibres relies heavily upon *in vitro* assays. However it is not clear how relevant such *in vitro* systems are as measures of the *in vivo* response. (For example, can *in vitro* systems provide a satisfactory mimic of the effects of tissue residence upon a fibre?) There is a definite requirement for a critical appraisal of the relevance of *in vitro* and other short-term assays to the biological response to fibres. The situation is in stark contrast with, for example, the understanding and use of *in vitro* and other short-term assays for genotoxic carcinogens, a comparison which demonstrates how neglected research into the mechanisms of action of fibres has been. The key research questions relating to mechanisms of action are as follows, in no order of priority:

What are the key biochemical events at the cell/fibre interface? (For example: What is the magnitude of duration and extent of free radical release? What membrane moieties are involved - and are they different for different fibres? What are the critical chemical characteristics of the fibre surface?)

To what extent does the biological coating or opsonisation of a fibre modulate the magnitude or nature of the biochemical events at the cell/fibre interface?

How does dissolution of a fibre alter its biological activity?

What are the important and relevant inflammatory mediators released following contact of fibres with extracellular fluids and/or cells such as macrophages, epithelial cells, mesothelial cells, etc?

How does sustained insult from fibres affect the magnitude and the outcome of the inflammatory response?

How is cytokine production affected by fibre exposure, and which cytokines are involved?

How does genome damage occur and what (if any) is its significance?

In what ways do fibres alter gene expression; which genes are affected?

Can oncogene activity be detected after realistic fibre exposures *in vitro* or *in vivo*, and which oncogenes are expressed?

How do different types of cell differ in their response to, and interaction with, fibres?

OPPORTUNITIES FOR RESEARCH AND DEVELOPMENT

There is a need to characterise the initial response to fibre exposure, that is the key biochemical events at the cell/fibre interface, including the role of phagocytosis, and to determine how (if at all) these are affected by the dimension of the fibre. The key factors in terms of fibre composition that affect mechanisms of action should also be examined since fibres may be modified and contaminated with use.

The tissue residence of a fibre may influence its biological activity, in terms of both protein adsorption (or other biological coating) and possible effects of progressive dissolution. The 'metabolism' of a fibre may be different when it is fully incorporated within a cell, in comparison to when there is a significant extracellular component. A better understanding of the influence of tissue residence on fibre activity is of particular importance to the establishment and use of short-term assays for fibre toxicity, since such assays should accurately mimic the persistence and activity of the fibre over the long-term *in vivo*.

There is an important need to examine critically the hypothesis that fibre toxicity is mediated by free radicals, probably by including tests of the relevant null hypotheses rather than solely by further descriptive and corroborative studies. This is regarded as a valuable and attainable target in the short- to medium-term, and is particularly pertinent now that the technology exists to examine most aspects of the proposed roles of reactive oxygen intermediates in the disease process. Such a critical examination should include both the injurious effects of free radicals and the key gene regulatory consequences.

2.2 STUDIES IN EXPERIMENTAL ANIMALS

GAPS IN KNOWLEDGE AND RESEARCH NEEDS

The following gaps in existing knowledge have been identified and research needs are ranked in order of priority:

1 Fully characterised standard reference fibres are recognised as being of great importance in order to advance research on the development of *in vitro* assays and studies of mechanisms *in vivo*. Ideally the reference samples will cover a range of surface properties and fibre sizes. Selective use of the reference fibres will enhance the comparison of results between laboratories; this will aid

both hazard and risk identification and the examination of mechanisms of fibre toxicity. In developing the reference materials the sensitivity of the various toxicological or other outcomes to small changes in the properties of the reference fibres will need to be taken into account; it will be important to ensure sample homogeneity as well as close control over specifications.

Current understanding of the mechanisms of fibre toxicity does not allow an adequate prediction of toxic effects, thus a broad range of research is to be encouraged; there is insufficient knowledge for research to be restricted to any specific areas of investigation.

Currently there is very little coherent information on the dose-response relation for fibre toxicity following inhalation, or the relation between exposure and tissue dose. The validity of the experimental models used is not well understood; for example it is not known what constitutes an excessive dose for the purposes of evaluating possible effects in humans. The importance of exposure intensity, or rate of dosing, as well as cumulative total dose should also be investigated. These are important issues, particularly in terms of making human health risk assessments.

2 The use of intermediate and early histological and molecular end-points associated with eventual tumour development may be possible for risk assessment; in this context the use of biomarkers should be investigated as this might obviate the need for long-term experiments.

Further research is needed on the validity of inter-species extrapolation. Such studies are needed both for the comparison of mechanisms of toxicity between species and for human risk assessment purposes.

Investigations into the effects of fibre durability *in vivo* should be undertaken (see also above) and the importance of dosimetry and the significance of overload phenomena should be studied. There is a need to establish a set of reliable validated models. Such models could be used to predict the characteristics of novel fibres. There are currently many models of fibre durability and these have to be validated *in vivo*. Studies are also needed to establish whether the calendar time durability of a fibre or its durability as a proportion of lifespan is the more important factor. Such studies can only be carried out *in vivo*.

3 The importance of co-exposures and synergistic effects should also be investigated.

OPPORTUNITIES FOR RESEARCH AND DEVELOPMENT

The lack of reference fibres is seen as a significant impediment to progress in research. A bank of standard reference fibres would be a great asset to all experimental studies, not only those in experimental animals, but also *in vitro* studies of fibre durability and persistence, and of mechanisms of toxicity. The reference bank should comprise a number of specified fibres, which may include some existing fibres as well as novel fibres produced specifically for reference purposes. The physicochemical properties of all standard reference fibres, as well as their dimensions and biological activity *in vitro* and *in vivo* will need to be characterised and standardised. Fibres with similar composition and different dimensions and fibres with similar dimensions and different compositions will need to be included. Whilst recognising that it is still not clear which dimensions are the most relevant to toxicological outcome, fibre dimension will be a critical factor in choosing reference fibres. Although recent advances in technology mean that the production of single size fibres in experimentally significant quantities is a realistic prospect, the need to produce large (kilogram) quantities of fibres with standard characteristics will require some technological development. Where the composition requirements cannot be met by currently available fibres, new materials will need to be synthesised. It will also be necessary to identify how and by whom the fibres will be produced, where they will be held and the conditions, if any, for their release to researchers. The development of such a facility will undoubtedly provide an impetus for new research initiatives and its establishment will incidentally foster collaboration between biologists and materials scientists.

Another area where major research efforts would be appropriate is in establishing the parameters of tissue dosimetry, particularly in relation to hazard and risk. This may be aided by the establishment and validation of a suitable dosimetry model, whereby inhalation exposures can readily be compared with those by other routes, which may prove to be more convenient for investigative purposes. Such a model must also take into account the effect of solubility on tissue dosimetry. The means by which the model can be extrapolated between species should also be established. This will necessitate the determination of the relative importance of calendar time and the proportion of lifespan as determinants of the pathogenic response. Characterisation of the biological effects of the standard reference fibres described above will be essential.

The combination of the judicious use of standard reference samples and the development of suitable models of dosimetry by the inhalation route will provide

a useful baseline standard against which judgements about the appropriateness of experimental dosing regimes can be made. This is particularly important in risk assessment if data from *in vitro* and other short-term test systems are to be used successfully. The progress of such a programme will require close collaboration between a variety of scientific disciplines.

2.3 EPIDEMIOLOGY AND RISK ASSESSMENT

There are two groups of fibres to consider: asbestos and existing man-made mineral fibres, for which data are available on effects in humans; and the newer fibres about which little is known in terms of human health effects.

GAPS IN KNOWLEDGE AND RESEARCH NEEDS

Asbestos

Asbestos is known to cause *mesothelioma, asbestosis* and *bronchial carcinoma*. Only continuing epidemiological analysis will establish whether or not controls have been set appropriately and whether compliance with the control measures has been satisfactory. These issues can be addressed in different ways for the different diseases caused by asbestos as indicated below in no order of priority:

> The incidence of *mesothelioma* is rising steeply in the UK and a recent analysis has suggested that total numbers will continue to rise until 2020. Monitoring of the incidence of and mortality from mesothelioma should be continued and the histology of cases should be verified.

It has been suggested that exposure to vaccine contaminated with Simian Virus 40 (SV40) might be another cause of *mesothelioma* and thus confound trends in its occurrence. The possible role of SV40 in the induction of mesothelioma could be investigated in the course of a case-control study, provided adequate methods were available to assess exposure to the vaccine in both cases and controls. This would require collaboration between laboratory based researchers and epidemiologists.

The number of deaths from *asbestosis* is apparently rising currently. A proportion of deaths attributed to asbestosis are known to be incorrectly certified. However, there may also be under-reporting of some asbestosis deaths. Confirmation of diagnosis is therefore extremely important.

Although it is recognised that most long fibres will induce some fibrosis (collagen deposition) in the lung, this is not necessarily the same as clinically disabling fibrosis; agreement as to what constitutes a significant level of fibrosis has yet to be established.

There are several cohorts of asbestos exposed workers currently under investigation. These cohorts, particularly those with relatively low exposure, can give information on the incidence of *bronchial carcinoma* among people exposed within the limits currently in force. Studies on cohorts with exposure to chrysotile but not crocidolite or amosite may have important implications both for understanding carcinogenic mechanisms and for the management of chrysotile in buildings. It is therefore important to continue the follow-up of the asbestos-exposed cohorts. Another need is to find out to what extent chrysotile is contaminated by other fibres, such as tremolite.

Man-made mineral fibres

There is still some residual uncertainty concerning whether the production and use of man-made mineral fibres poses a hazard to health. In order to answer this question the International Agency for Research on Cancer (IARC) cohort study should be continued, but there is little need to set up additional cohort studies on these particular fibres.

To facilitate the interpretation of epidemiological studies it would be advantageous to examine the lung burden of fibres in the members of the IARC cohorts, particularly those exposed to rockwool and slagwool.

Novel fibres

It is important to be able to make a reasonable evaluation of the likely health consequences of exposure to novel mineral fibres for which there are few or no epidemiological data available. An attempt is made below (Table 1) to present, in summary, the available human data against which any evaluation of novel fibres based on laboratory experiments or structure-activity could be tested. It is unlikely that it will be possible to extend this database any further in the foreseeable future

TABLE 1: EVIDENCE FOR FIBROSIS, CARCINOMA AND MESOTHELIOMA AMONG HUMANS EXPOSED TO MINERAL FIBRES

	Fibrosis	Carcinoma	Mesothelioma
Crocidolite	+++	+++	+++
Amosite	++?	++	++
Tremolite	?	?	+++
Chrysotile	+	?0	?0
Rockwool	0	?0	0
Slagwool	0	?+	0
Glasswool	0	0	0
Erionite	?	0	+++

+++ strong evidence ++ some evidence + weak evidence
0 no evidence ? uncertain

Some studies might contribute towards the evaluation of possible adverse health effects of novel mineral fibres and these are listed below in no order of priority:

A study is needed to establish whether the risk of lung cancer increases in the absence of fibrosis in people exposed to asbestos. This could be very important in evaluating new substances, since if fibrosis is a prerequisite for mineral fibre induced lung cancer, and a particular fibre does not produce fibrosis, then its use would cause less concern.

Short-term outcomes could be used to assess likely health consequences of novel fibres. The most useful end-points are considered to be cellular profiles and cytokines measured in bronchial lavage specimens.

Other biomarkers that might be used as short-term effect markers should also be developed and investigated. Increased serum oncogene levels have, for example, been reported in asbestos exposed workers; these were found before the clinical diagnosis of cancer in these cases. Such an initiative also will require collaborative studies involving experimental and epidemiological disciplines.

The rate at which fibres disappear from lavage samples may also be informative, since if one type of fibre disappears more rapidly following exposure than do others it might be considered to be potentially less harmful. However, the disappearance of intact fibres from bronchoalveolar tissue, rather than indicating that they are short-lived, could also indicate that the fibres have migrated into the interstitium, where they might in fact be more harmful. Finding frayed or fragmented fibres in bronchoalveolar lavage fluid would perhaps be more informative.

OPPORTUNITIES FOR RESEARCH AND DEVELOPMENT

Epidemiological evidence arising from the use of existing fibres should be used as far as possible to evaluate the potential hazard of novel fibres. One question that it is essential to answer is whether or not fibrosis is a prerequisite for lung cancer. Studies in New Orleans and in crocidolite miners in South Africa demonstrate an association between fibrosis and lung cancer, which is supported by studies in experimental animals (Sluis-Kremer & Bezuiderhout, 1989; Davies & Cowie, 1990; Hughes & Weil, 1991). However, there is still a need to clarify what degree of fibrosis and what extent of lung fibre-burden are necessary for

lung cancer. These questions can be addressed using evidence arising from human exposure to asbestos.

A useful, relatively short-term study would be to measure the persistence, after inhalation, of fibres in the human lung. This could potentially be of benefit to hazard assessment for novel fibres.

The apparent increase in mesothelioma trends in the UK should be validated by histological review of the cases, which could be incorporated in a case-control study. Such a study should also investigate which occupational or other activities are responsible for the increasing incidence.

Case-control studies, in which detailed occupational histories are collected, should also be set up to check the effectiveness of current controls on exposure to mineral fibres. Such studies could include assessments of fibre burdens in the lung, particularly in mesothelioma cases, since this might give early warning of hazards from other fibres that have not previously been linked with the disease. A study of the presence of excess non-asbestos fibres in the lungs of cases as compared to controls would be valuable as a possible early indication of a hazard from these fibres. It is, however, recognised that in different occupations there seem to be different associations between lung fibre-burdens and mesothelioma rates. Furthermore, lung fibre-burdens generally represent recent exposure, which may be quite different from the exposure that provoked the tumours.

3 Recommendations

3.1 PROPOSED RESEARCH PROGRAMME

Based on the research priorities described in the previous section, several overall priorities have been developed into proposals that could form the basis for a national programme of research. The aim of the research programme proposed below is to facilitate the hazard assessment of novel and existing fibres in both the short- and the long-term. Four key areas are identified as follows:

Standard reference fibres
Tissue dosimetry
Mechanisms
Epidemiology

At the outset of this project it was apparent that there are two important issues that drive current research into mineral fibres. The first is the assessment of the human hazard of both existing mineral fibres and novel fibres that have been developed to replace or supplement existing fibres. A human hazard assessment for these fibres is an immediate necessity and has to be undertaken using available knowledge. While it is accepted that the present understanding of fibre characteristics, toxicity and mechanisms of action may be far from adequate for an accurate hazard assessment, only research of an applied nature, conducted in the short-term, will make a difference to hazard assessment in the near future.

The second issue, which requires long-term basic research, is a better understanding of the mechanisms whereby mineral fibres may cause fibrosis, mesothelioma and lung cancer. The two issues are related, since a better understanding of mechanisms will lead to better hazard assessments and ultimately better risk assessments. This second element of the research programme should attract not only those interested in fibre carcinogenicity, but also those with an interest in the mechanisms of carcinogenicity *per se*.

The proposed research programme therefore comprises two elements: applied research to be conducted over the short-term to answer questions specific to the best immediate assessment of hazard from existing mineral fibres or fibres currently under development; and long-term basic research to establish the mechanisms of action of mineral fibres.

APPLIED RESEARCH

STANDARD REFERENCE FIBRES

The setting up of a reference bank of standard fibres should be given a high priority. This has been recognised for some time as an essential foundation for any comparative research on fibres, yet little has so far been done to enable such a reference bank to be established. Any action that might facilitate this goal such as, for example, a workshop to promote a better discussion of the specific requirements and to put forward considered and costed proposals should be supported.

Although it is recognised that to establish such a fibre bank will be technically difficult and expensive, it is nonetheless achievable within the short-term given the appropriate resources and collaboration, particularly between potential funding bodies and between academia and industry. Easy access to standard fibres is a prerequisite for effective research in all fields of relevance to the improvement of human hazard and risk assessment.

TISSUE DOSIMETRY IN RELATION TO HAZARD AND RISK

Much of the research into fibre dissolution and persistence in *in vitro* systems and *in vivo* in different tissues and species is of a basic and long-term nature and is outlined below. However, *the identification and validation of a limited number of generally accepted* in vitro *assays of fibre dissolution should be encouraged* in the short-term. Although studies in this field can be, and have been, undertaken without access to standard reference fibres, the availability of such fibres would certainly facilitate comparisons between studies and the validation of results.

STUDY OF MECHANISMS OF CARCINOGENICITY

Mechanistic studies that should be achievable in the short-term include *an evaluation of the association between short-term markers for fibre toxicity and long-term effects.* Studies into mechanisms of action of mineral fibres can also progress without access to standard reference fibres. However, the value of mechanistic studies (both short-term, as outlined above and long-term, described below) would be greatly enhanced if they could be undertaken using fibres with well characterised physicochemical and toxicological properties, so that the relevant contributions of those properties to the toxic effects of fibres could be assessed.

EPIDEMIOLOGY

Confirmation of the reports that mesothelioma incidence is indeed increasing in the UK should be given a high priority, since the potential public health implications of recent findings are serious. Although epidemiological studies on the aetiology of mesothelioma belong to basic research, validation of the apparent trends is achievable in the short-term.

BASIC RESEARCH

STANDARD REFERENCE FIBRES

The establishment of a bank of standard reference fibres falls into the category of applied research as described above. Access to standard fibres will nonetheless facilitate all areas of research including basic research into fibre durability, mechanisms and toxicity. The maintenance of such a reference bank has long-term implications for resources and funding.

TISSUE DOSIMETRY IN RELATION TO HAZARD AND RISK

There are a number of basic issues to be addressed that will require a longer-term approach. These include *fibre persistence (clearance and durability)* in vitro *and* in vivo*, the effect of tissue residence on the characteristics and activity of fibres and an examination of the importance of dose and dose rate. Species differences in response to fibres and the validity of extrapolation between species, including humans, should also be investigated.* Many of these studies are multidisciplinary in nature and therefore collaborative studies are encouraged.

STUDY OF MECHANISMS OF CARCINOGENICITY

Studies on the mechanisms of fibre carcinogenesis will require a long-term approach and investment in basic research. Without a proper understanding of mechanisms it will not ultimately be possible to predict and therefore control risks from exposure to mineral fibres adequately. As described above, *epidemiological studies are needed to examine the association between fibrosis and lung cancer, and experimental studies should address cell-fibre interactions and critically examine the proposed role of oxidative stress in fibre carcinogenesis*. Despite considerable research on the mechanisms of fibre toxicity, there is no agreement on the mechanism or mechanisms for fibrosis, mesothelioma and lung cancer; hence innovative approaches are likely to be required. A multidisciplinary approach is clearly necessary and substantial support from groups involved in studying the basic biology of cancer is likely to be beneficial.

EPIDEMIOLOGY

Epidemiological studies should be undertaken to resolve outstanding questions about the aetiology of mesothelioma. To this end other factors, such as the role of SV40 virus, should also be investigated. *Epidemiological studies on the association of mesothelioma with occupation and lung fibre-burden should also be undertaken.* Although forming part of a basic research programme it should be possible to obtain useful results in the medium-term from adequately resourced studies.

3.2 CONCLUSIONS

The views expressed in this document are those of a group of experts in fibre toxicity expressed through two workshops. There is clearly much to be done before knowledge of mechanisms of fibre toxicity and carcinogenicity is adequate to predict human hazard from existing or novel mineral fibres. The outline of research needs and priorities described herein is intended to direct research to improve the ability to predict hazard in an area that is both of considerable importance to human health in the UK and a substantial scientific challenge.

Progress in the past has been hampered by a fragmented approach from funding agencies, which have supported small initiatives with inadequate co-ordination between research groups. There is now an opportunity to make better use of existing research funds through collaborative and properly co-ordinated research initiatives.

Clearly there is considerable overlap between those elements of the research programme that will contribute to resolution of the immediate needs for hazard assessment and those that constitute basic research. It is therefore recommended that a co-ordinated national research programme on mineral fibres, involving all interested parties undertaking both applied and basic research, should be established.

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Appendix:

REVIEWS OF CURRENT RESEARCH ON MINERAL FIBRES AND IDENTIFICATION OF GAPS IN KNOWLEDGE

HEALTH AND SAFETY EXECUTIVE: EXPERIMENTAL STUDIES

M Meldrum

INTRODUCTION

A fundamental requirement, from the regulatory perspective, is to be able to predict or identify accurately the health hazards and dose-response characteristics of existing and new fibres. Such information, together with information on the nature and extent of exposure in the workplace, is needed for risk assessment purposes and for guiding decision making activities in relation to standard setting and other occupational control measures. The need for accurate information on the health hazards of fibres is essential to avoid both unnecessarily stringent regulatory control measures and another asbestos tragedy.

As illustrated by the protracted regulatory activities concerning the classification of carcinogenicity of man-made mineral fibres (MMMFs), there is a certain lack of scientific agreement concerning the process of hazard identification for fibres (even for fibres with a large toxicity database). A particular concern is that with advances in manufacturing technology, together with the decline in the use of asbestos, fibres with entirely novel physical and chemical properties are now coming into commercial use. The existing database of hazard information for asbestos and MMMFs may be of uncertain relevance in predicting the hazards of new fibre types.

To address such problems there is a need to develop a rational fibre toxicity testing strategy. This requires a harmonised approach to the interpretation of all data perceived to be relevant to hazard assessment. Achievement of these aims is limited by the lack of agreed test method protocols. However in recent years the scientific community has made significant progress in this area, and at a conference in Paris in February 1994 (Bigon *et al.*, 1995) a co-operative inter-laboratory programme was initiated to develop agreed test methods for fibres.

Ideally, a testing strategy for fibres would be based on predictions made on the basis of the physical and chemical characteristics of the fibres, together perhaps with the results of a carefully selected number of short-term *in vitro* tests. Short-term animal tests may also be needed, but the benefit of a well designed fibre toxicity testing strategy would be to reduce or avoid altogether the need for long-term animal studies. Dose-response characteristics could be defined on the basis of estimations derived from mathematical modelling of deposition and clearance rates, which is an area of research showing much promise. These goals are seen as desirable not only from the animal welfare point of view, but long-term animal inhalation studies are costly, time consuming, and do not always yield data which can be readily interpreted. It is uncertain whether these aims will ever be realised in practice. However, as a step towards these aims the research priorities for fibres can be broadly grouped into three areas:

(i) Defining the relationship between physicochemical properties and toxicity

(ii) Understanding the mechanisms of fibre-induced toxicity

(iii) Development of short-term predictive tests.

RELATIONSHIP BETWEEN PHYSICOCHEMICAL PROPERTIES AND TOXICITY

At the moment, it is not possible to make an accurate prediction of hazard based on a knowledge of the physicochemical properties of a fibre alone, although some tentative conclusions could be drawn based on current knowledge. For example, the potential to cause chronic lung damage will be less with relatively soluble fibres than with more durable fibres, and thus an index of solubility in physiological solution would be a useful parameter in predicting hazard. The solubility behaviour of a fibre in physiological solution may not always accurately reflect events in the lung, (for example due to deposition on the fibre surface of various materials by macrophages) and therefore solubility studies conducted with fibres incubated with mammalian cells could provide a further indicator of durability *in vivo*. Mineral fibres may show differences in dissolution rates under different pH conditions, and therefore information on solubility at different pHs is needed to allow for the pH differences in intra- and extracellular fluids in the lungs and pleural tissues. A concerted effort toward standardising the test methods for such studies and developing a set of criteria for evaluation of the results is needed. Further research into understanding fibre solubility and how this relates to chemical composition is judged to be of high priority.

Given that other less well understood fibre properties are probably important determinants of toxicity, it is doubtful whether a single numerical index of solubility would be adequate to predict fibre toxicity. However, in conjunction with other relevant test data, an index of fibre solubility would be important in the context of a fibre testing strategy. Research is needed to identify other physicochemical properties of fibres which could be exploited as potential indicators of toxicity. The ability of fibres to generate oxygen containing free radicals when incubated with macrophages and mesothelial cells may provide one such index.

The interpretation of studies designed to explore the significance of particular fibre properties would be aided by the ready availability of size-selected fibres, in order to reduce confounding effects caused by dimension differences. Water sedimentation has been employed to separate long and short amosite fibres, and sieving through wire mesh has been employed with less success in size-separating chrysotile. Research into developing non-destructive techniques for size-separating fibres is considered to be potentially very useful.

MECHANISTIC STUDIES

Success in developing short-term tests for predicting the hazards of inhaled fibres depends on understanding the mechanisms of toxicity for each of the end-points of concern. It is unclear whether a single unified mechanism of toxicity could be identified which would account for fibrosis and cancer in the lungs as well as for mesothelioma. A great deal of progress has been made in understanding the development of fibre-induced pulmonary fibrosis at the biochemical and cellular level. A number of cytokines have now been identified which are thought to be involved in fibrogenesis and cell proliferation. Animal studies investigating cytokine gene expression in macrophages show great promise in helping to elucidate some of the early responses to inhaled fibres, but the importance of species-specific effects still needs to be addressed. Research into these areas is perceived to be of considerable value both for understanding the early stages of disease development and also for providing a rational basis for designing short-term predictive test methods.

There are still uncertainties concerning the nature of fibre translocation mechanisms following deposition in the lungs. Although some studies with radioactive mineral fibres have been reported, there may be some value in further research using size-selected radioactive fibres to monitor the time

course and path of translocation following inhalation exposure. Such studies might be of particular value with radiolabelled synthetic organic fibres.

Less well understood are the processes leading to fibre-induced carcinogenesis. There would appear to be an increasing number of studies looking at oncogenes and tumour suppressor genes in human and rodent mesothelioma cells, but such studies have not provided a hypothesis to account for the development of fibre-induced mesothelioma. It is unclear whether such studies could contribute to the design of short-term predictive tests, or reliably inform on fibre hazard. Therefore, while being of considerable interest, such research is not considered to be of high priority relative to other areas.

There are still uncertainties as to whether direct genotoxic effects are involved in fibre-induced carcinogenesis, or whether repeated cycles of cell damage and cell proliferation lead to neoplastic transformation without invoking any specific genotoxic effect. The resolution of such issues may provide a logical basis for developing short-term tests and information on the relevant markers for investigation.

DEVELOPMENT OF SHORT-TERM PREDICTIVE TESTS

It would be very useful to be able to make reliable predictions of the hazards of inhaled fibres, using short-term tests, ideally *in vitro*. However, research related to other areas of toxicology, for example neurotoxicity and reproductive toxicity, has so far failed to lead to *in vitro* test methods which would be acceptable to regulators as predictive of *in vivo* hazard. This may reflect the complexities of the relationships between target cells and the organs and tissues involved. As noted above, an understanding of the mechanisms of fibre-induced toxicity which occur *in vivo* is needed to form the basis of well designed short-term predictive tests. Considerable progress has been made in understanding fibre-induced pulmonary fibrosis, and it may therefore be possible to design tests to measure end-points known to relate to fibrogenic hazard. For example, measures of the release of cytokines and inflammatory mediators from macrophages and lung fibroblasts incubated with fibres, and measures of the ability of fibres to provoke epithelial cell detachment from their substrate *in vitro*, might be appropriate indices of hazard.

Until more progress has been made with mechanistic studies, short-term tests in animals may provide a more fruitful line of research than *in vitro* tests, and may yield more meaningful results. Studies on cell proliferation rates in different tissues (e.g. airway epithelial cells, subpleural and pleural tissues) following

inhalation exposure or intratracheal exposure may be useful. Appropriately selected positive and negative control fibrous dusts would be needed to help in the interpretation of the results.

Inhalation studies with fibres are technically difficult, and probably few laboratories have the necessary skills and experience to carry out such work. However this is an important area if short-term animal inhalation studies are to be promoted within the context of a fibre toxicity testing strategy. Also, little is known about the relative values of nose-only compared with whole body exposures to fibres in rat inhalation studies. Research into developing the optimum experimental techniques for such studies may be needed.

Warheit and Johnson (1990) suggested that the development of pulmonary disease is correlated with four interdependent factors which include the capacity of the inhaled fibre to produce ongoing (i) cytotoxicity, (ii) inflammation, (iii) reduced macrophage function and (iv) biopersistence of the inhaled material in the lung. These authors suggested that the investigation of these factors following short-term inhalation exposure may be used to predict the development of pulmonary toxicity. However, comparison of the results of long-term inhalation and short-term studies with the same fibre (aramid fibres) suggest that it is difficult to make such predictions. Warheit *et al*. (1992) exposed rats for three to five days to up to 1000 fibres per millilitre (f/ml) of aramid fibres. An acute inflammatory response was observed which was reversible one month post-exposure, and by three months there was no histopathological evidence of pulmonary lesions. In contrast, a spectrum of chronic pulmonary damage was observed in rats exposed for up to two years at concentrations of 25 f/ml and above (Lee *et al.*, 1988). The contrast between these findings suggests a need for caution in making predictions about health hazards of novel fibres based on short-term tests alone. The repeated pulmonary deposition of cytotoxic fibres, even at relatively low airborne concentrations, may be able to produce a low level of chronic lung damage. More studies to explore the predictive value of short-term inhalation studies are needed.

CURRENT RESEARCH

The HSE has actively supported experimental research into many aspects of fibre toxicology. Notable amongst these research efforts is the work conducted at the Institute of Occupational Medicine (IOM) under the auspices of the Colt Foundation. The work at the IOM has allowed comparisons of the toxicity of up

to 12 different fibres in intratracheal and intraperitoneal studies, and has also measured the *in vitro* solubility of these fibres. Inhalation carcinogenicity studies have been conducted on those fibres for which sufficient quantities were available. Cell biology studies and inflammation in the mouse peritoneal cavity have also been investigated. The body of data which will eventually be available should be instrumental in identifying which fibre properties most closely correlate with toxicity, and should assist in the design of short-term predictive test methods.

RESEARCH NEEDS

An HSE seminar, held in 1995 to discuss the value of *in vitro* genotoxicity and molecular biology studies with fibres should help to decide on the most effective use of resources. It is hoped that this IEH report will also be of use to the HSE in evaluating the potential uses of such studies. At this point in time, it would seem that the most promising areas of research which could realistically yield useful information are investigative studies to identify which fibre properties (including solubility) most closely relate to hazard, and research into developing predictive markers of pulmonary and pleural toxicity from short-term animal studies with intratracheal and inhalation exposure. Mechanistic studies are also perceived to be important in improving our understanding of the underlying toxicological processes involved in fibre-induced lung and pleural disease.

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HEALTH AND SAFETY EXECUTIVE: EPIDEMIOLOGICAL STUDIES

J T Hodgson

CURRENT KNOWLEDGE

The epidemiological evidence, which is largely based on the observation of occupationally exposed cohorts, has established a range of facts about the health effects of asbestos. These facts are much more securely established qualitatively than quantitatively.

Exposure to asbestos increases the risks of three distinct disease outcomes: asbestosis, mesothelioma and lung cancer.

Dose-response curves have been demonstrated in some populations for all these diseases, but the variations in risk coefficients are wide. Fibre type is almost certainly important.

The rates of mesothelioma increase very steeply with time from first exposure. This complicates the estimation of the contribution of exposure intensity, since differences in the exposure timing produce large effects in relation to the reliably detectable differences in exposure intensities in a typical study population.

The joint effects of smoking and asbestos exposure on lung cancer are more than additive; this has implications for the possibility of estimating the effects of asbestos itself.

The fundamental difficulty with the interpretation of these data is the absence of extensive reliable exposure data (compounded by the specific difficulties of estimation mentioned above). This situation is unlikely to change, and it is difficult to see that further epidemiological work on occupational cohorts will resolve the major remaining uncertainties. This is more likely to arise from a better understanding of mechanisms derived from experimental studies although an important touchstone for any theory of mechanism is that it

should be consistent with the established epidemiological facts. This is not to say that nothing of further value will emerge from continued observation of asbestos exposed cohorts. The time dependence of risks is an important, and often neglected feature of follow-up studies, and continued follow-up may help establish whether relative risks are determined, for example, by cumulative exposure alone, or whether they fall after the cessation of exposure.

If there is little to be learned from further follow-up of cohorts of workers with (more or less) well-defined exposure, this may not be so for groups with sporadic exposure. Analyses of series of mesothelioma cases (including the HSE Mesothelioma Register) suggest that such cases may outnumber those due to 'full time' asbestos exposure by a substantial margin. The difficulties of exposure measurement for such groups are even greater than for the 'full-timers' discussed in the previous paragraphs. Can case-control studies using lung fibre-burden measurements provide useful information on this group? A number of studies have demonstrated a relationship between asbestos fibre lung burdens and mesothelioma, but the different clearance times of different fibre types from the lung considerably complicate the interpretation of such observations.

One important current issue for HSE is the interpretation of the trends in mesothelioma death rates as reflected in death certificates mentioning mesothelioma. These data show that the rates of mesothelioma in males have increased consistently and substantially for each birth cohort from those born at the end of the last century to those born around 1940 and around 1945. The rates then fell, by about 30% for those born around 1950 and about 40% for those born around 1955. There are no meaningful data for cohorts born more recently than this.

There are two general lines of interpretation for these observations. The pessimistic interpretation emphasises a further feature of the data, which is that the age-specific rate curves for each birth cohort appear to fit a fixed relative risk model for rates at different ages (in other words for every cohort the ratio of rates at two given ages is constant). If it assumed that these relationships will continue to hold into the future, the implied total numbers of deaths for the 1940 and 1945 cohorts would be over 1% of total mortality, and even those for the 1955 cohort will be over 0.5%. These predictions imply large numbers of asbestos related deaths, even in the most recent cohorts, most of whose working lifetimes will have fallen in a period when the dangers of asbestos were thought to be well understood, and exposures, at least in asbestos manufacture, were

being controlled to below one fibre per millilitre (f/ml). If these conditions will generate 0.5% of total deaths from mesothelioma, the pessimistic interpretation runs, what grounds are there for expecting substantially improved outcomes for later cohorts?

The alternative interpretation emphasises the observed reduction in risk for the two most recent cohorts (which, after all, represent almost a halving of risk between the 1945 and 1955 cohorts), and argues that this demonstrates that the efforts to control asbestos exposure have indeed had a substantial effect on the resulting mesothelioma rates. Given this fact, it is unreasonable to assume that the relativities of risk of different ages will hold for these cohorts in the same way as for earlier cohorts. The accumulating data will eventually show which of these interpretations is nearer the truth, but the uncertainty creates a need for HSE to seek the earliest possible evidence to help decide the issue.

Another important issue for the interpretation of these data is the possibility of changing diagnostic criteria. If cases of mesothelioma are being identified with increasing completeness (or if there is an increasing trend of false positive diagnoses), the true trends will be exaggerated. A closely related question is that of the 'natural' background rate for mesothelioma. Commonly quoted figures for this are one or two deaths per million person years, implying about 50-100 annual deaths nationally.

In addition to the birth cohort analyses discussed above, analyses of the occupations recorded on mesothelioma death certificates show some features of concern. Data that have been prepared for publication in the Occupational Health Decennial Supplement (Drever, 1995) show a wide range of relative risks between occupations, but in no reasonably large occupational group is the number of deaths low enough to be consistent with the assumed background rate. Among the 30 job groups providing more than 1% of total 'all cause' deaths (in males) the lowest risk group for mesothelioma is farmers, with a PMR of 0.25 (95% CI, 0.18 - 0.35); an occupation with mesothelioma mortality of two per million would have a PMR of 0.05, one fifth of the observed PMR for farmers. No job group shows a PMR significantly lower than that for farmers.

RESEARCH NEEDS

The HSE is reviewing these issues and how they might be addressed by further research. A case-control study of younger mesothelioma cases is an obvious option, but there are important methodological choices to be made concerning the choice of control series and the range of occupational and exposure data to be gathered.

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DEPARTMENT OF THE ENVIRONMENT: POLICY AND RESEARCH NEEDS

L M Smith

DEPARTMENTAL OBJECTIVES

The Secretary of State for the Environment has declared that one of the objectives for the Department of the Environment (DoE) is to maintain and improve the quality of life for everyone by promoting sustainable development and making legislative and administrative arrangements to ensure protection of the environment. DoE has the responsibility within Government for policy and controls on hazardous materials in the non-occupational environment. This includes protection of both the natural and man-made environments and protection of the public from exposure to hazardous materials through environmental pathways.

Departmental policies encompass measures to protect all aspects of the environment including air, water, land, all living things and the man-made and urban environments. The Department also has responsibilities for land use planning, minerals extraction, housing and the construction industry.

CONTROLS ON HAZARDOUS MATERIALS

The Department has responsibility for a range of legislation and administrative controls to prevent or limit hazardous materials entering the environment. These include integrated pollution controls regulating emissions to air, water and land, controls over the disposal of waste and regulation of diffuse sources of hazardous materials in products such as construction and consumer products. Hazardous materials may also be controlled through European and British Standards for products. The Department also publishes a range of advice for the public on hazardous materials.

DEVELOPMENT OF POLICY

Policy development depends on a proper assessment of the risks which hazardous materials pose to people and the environment. Control measures will only be introduced if they can be justified; however in many instances DoE will adopt a precautionary approach if the risk of environmental damage is suspected but cannot be proven. To carry out such risk assessments DoE needs accurate information on the hazardous properties of materials and their movement and fate in the environment, with particular emphasis on their persistence, effects on human health and the environment and the routes and levels of exposure. Risk management and risk reduction measures can then be devised.

ASBESTOS

Asbestos is a particular problem in environmental terms because as well as being a known carcinogen, it presents a cumulative risk to health and is persistent in the environment, retaining its fibrous nature almost indefinitely. Although exposure can be well controlled in the workplace, it is much more difficult to manage in the non-occupational environment. Particular problems are the legacy of its ubiquitous use in buildings, particularly homes, and the widespread use of asbestos products such as asbestos cement. The public are exposed due to general background levels and more specifically from undertaking do-it-yourself (DIY) work, through uncontrolled releases into the environment during demolition work and due to fires in which asbestos materials can become friable and debris may be spread over wide areas. In addition, and most significantly, there are problems and long-term costs associated with the disposal of asbestos waste.

The current legislative regime:

- prohibits the import and use of blue and brown asbestos and certain materials containing white asbestos;
- requires measures to contain and manage asbestos in existing buildings and products;
- controls the disposal of asbestos waste which must be contained and disposed of in specially licensed sites;
- controls the development and use of land contaminated with asbestos.

The Government's current policy, announced by the Minister for the Environment in September 1991, is to seek agreement in the European Community for a complete ban on the import or production of asbestos materials with derogations for specific uses. Remaining uses of asbestos should be limited to essential cases where less hazardous substitutes are not yet available; its use should be based on an agreed assessment of the risks and costs during production, use and disposal. The Government's aim is to see this policy introduced throughout the community as part of its strategy to assess and where possible reduce the potential health and environmental impact of hazardous substances.

This still leaves the legacy of thousands of buildings containing asbestos materials and products. The policy here is set out in the DoE publication *'Asbestos Materials in Buildings'* (Department of the Environment, 1991). This recommends that efforts should be made to identify all asbestos in buildings and manage it so that it does not pose a significant risk to the occupants. Asbestos in good condition should not be removed but should remain in place and be monitored. Asbestos materials in poor condition should be removed only if they cannot be contained. Special measures are also needed during the eventual demolition of buildings containing asbestos.

The Department actively discourages all use of asbestos products.

MAN-MADE MINERAL FIBRES

Man-made mineral fibres (MMMF) are ubiquitous in the environment; almost every home in the country contains such material as insulation for lofts, pipes and tanks, cavity walls etc. At present there is no restriction on the use of MMMF, but DIY products such as loft insulation do carry a limited health warning.

The present policy is based on the research into the levels of airborne fibres in the living spaces and loft spaces of typical homes carried out in the mid 1980s. In 1987 the Department of Health's Committee on Carcinogenicity carried out an assessment of the risks to health of non-occupational exposure to MMMF.

The Committee advised as follows:

> The levels of exposure to MMMF reported for living spaces resulting from domestic loft insulation do not pose a carcinogenic risk of any practical consequence to the health of residents.

The infrequent and short-term exposure to the higher levels of MMMF associated with DIY installations or disturbance of insulation do not pose a significant additional risk.

It would be prudent nonetheless for installers to wear an appropriate mask as recommended during installation.

The overall situation should be kept under review in the light of further developments in materials and type of installation.

The Department actively encourages the use of MMMF, including glass fibre and rockwool, for insulation of homes and other buildings as part of its energy efficiency programme and grants are available from local authorities. There is currently concern in the Department about the implications of any change in the classification of MMMF for marketing purposes.

OTHER DURABLE FIBRES

The use and prevalence in the environment of other durable fibres are small compared with asbestos and MMMF. The Department will need to keep under review both levels of emissions to the atmosphere and waste disposal policy.

RESEARCH NEEDS

The need for controls on fibres is dependent on authoritative assessments of the risks to health from exposure to fibres, and particularly dose-response relationships. DoE policy depends on such studies. DoE also supports the MRC strategy of funding high quality basic research into mechanisms by which fibres cause disease. In addition the Department acknowledges that there are a number of areas where information is lacking and new techniques for safe handling and disposal of fibres need to be developed. The major areas identified are outlined below.

ASBESTOS

development of cheap and effective methods for the destruction of asbestos fibres

further work on the risks of asbestos involved in fires

development of methods for the clean-up of land contaminated with asbestos

development of more effective control of the release of asbestos fibres during demolition of buildings

development of safe substitutes for the remaining uses of asbestos

MAN-MADE MINERAL FIBRES

clarification of the risks to health from exposure to MMMF

identification of the size and shape of fibres posing the greatest health risk so that products containing MMMF can be modified to reduce the risk to health

development of products which release fewer fibres to the environment

development of substitutes for MMMF which provide equivalent performance but less risk to health

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MECHANISMS OF DNA, MOLECULAR AND CELLULAR DAMAGE: AN OVERVIEW

*K Donaldson**

INTRODUCTION

The term respirable industrial fibres (RIF) is used here to include asbestos and man-made mineral fibres (MMMF) that are in the respirable size range, although most of the work alluded to has been carried out with asbestos. Clearly fibre length, fibre type, fibre durability and chemistry are the factors that currently dominate all research into the health effects of fibres and these are not discussed specifically here. However, any programme investigating the mechanisms of how fibres damage cells should take into account these important factors in the design and interpretation of the studies. There are now a number of fibre samples for which pathological potentials are known from well conducted animal studies (e.g. TIMA fibre repository samples, Colt Fibre Programme samples); the opportunity therefore exists for more rigorous examination, across a range of fibres, of some of the theories of mechanism mentioned below. It is to be hoped that future UK strategies for research into mechanisms of damage will benefit from utilisation of these well characterised samples.

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DIRECT AND INDIRECT DAMAGE

It is important to differentiate between direct damage caused to cells by RIF and indirect damage caused by the subsequent inflammatory response. Any research into the pro-inflammatory properties of RIF (not considered in detail in this paper) sheds important light on the 'damaging' effects of fibres.

DIRECT INJURY

This approach is now more refined than the mere measurement of cell death that characterised research in the early years. There are several main foci of research and the dominant paradigm is that fibres deliver a free radical injury to cells (see below for further discussion). There has been intensive study of the ability of fibres to produce free radicals at their surface, to detect evidence of the direct injury that this would produce and to describe the main factors involved (e.g. with iron). There have also been studies of the direct effects of fibres on chromosomes during mitosis that could lead to chromosome abnormalities and DNA damage. The cell surface receptors with which fibres interact when they make contact with a cell have been little studied; this is an important area for research, given the growth in understanding of cell surface receptors.

INDIRECT INJURY

In parallel with the growth of information concerning intercellular signalling *via* cytokines in the last decade, research into the bioeffects of RIF has shown an increasing emphasis on the consequences, for inflammation and pathological change, of contact between RIF and the cell. This approach tends to emphasise the ability of cells that have contacted a fibre to produce 'harmful' mediators that modulate the behaviour of a second (or third or fourth) cell type. This could arise from a permanent, genotypic change or as a phenotypic change in gene expression. It is now well accepted that the accumulation of inflammatory cells in tissue can lead, through the release of leukocyte products such as oxidants, proteases and cytokines, to tissue damage, altered architecture and pathological change. A major idea in the last five years has been that there must be altered gene expression for these events to occur and that an understanding of how RIF can switch on expression of particular genes, or mutate them, is the ultimate question.

IN VIVO MEASURES OF DAMAGE

INFLAMMATION

A range of methods is available to look at the injurious effects *in vivo* after exposure by both inhalation and instillation. Exposure by inhalation is both difficult, expensive and impractical for most cell biology or molecular biology end-points, which require much simpler systems. In some cases intratracheal instillation of fibres is carried out but the relevance of this non physiological deposition technique requires clarification. The technique of bronchoalveolar lavage (BAL) is used to sample the free cells of the bronchoalveolar space in rats exposed under controlled conditions to well characterised fibre clouds. Because of the central role of leukocytes in pathological processes this technique can yield valuable information and its use has greatly increased understanding of the events that occur following deposition of RIF (reviewed in Donaldson & Brown, 1993). Some work of this type has been carried out in humans but exposure, as always, is less well described and smoking is a complicating factor. By enumerating the macrophages and neutrophils in the BAL, inflammation has been found almost universally following inhalation of a range of asbestos and other fibres (Donaldson & Brown, 1993); the presence of inflammation could result from cell death, cell recruitment or cell activation/secretion.

It should be noted that in rat experiments in general the fibre number per ml is very high, often in the tens of thousands per ml. In humans who have been exposed to asbestos occupationally, at much lower fibre number per ml (<10), there is a trend towards more inflammation in the fibre-exposed groups but the confounding effect of smoking is a constant problem (reviewed in Donaldson & Brown, 1993). Inhalation of both asbestos and man-made RIF (e.g. Donaldson *et al.,* 1988; Warheit *et al.,* 1992) causes cell death which could be direct or indirect and is measurable as an increase in extracellular levels of the cytoplasmic enzyme lactate dehydrogenase. Interestingly, the macrophages that are retrieved from fibre-exposed lung are normally virtually 100% viable, suggesting either that RIF are not very cytotoxic to macrophages *in vivo* and the dead cells are epithelial cells, or that dead/dying macrophages are removed rapidly (perhaps by other macrophages). However, different methods of assessing cell viability may differ in their sensitivity: e.g. Trypan blue exclusion *versus* release of radioactive chromium. By examining the behaviour and activity of the cells in the BAL of fibre-exposed rats or humans and comparing them to those of normal BAL cells, it is possible to form hypotheses as to mechanisms of inflammation caused by

RIF exposure. This approach has been fruitful in suggesting that macrophages that have ingested fibres can release chemotoxins, oxidants, cytokines and proteases all capable of mediating inflammation and tissue damage (reviewed in Donaldson & Brown 1993; Donaldson *et al.,* 1993a).

Short-term inflammation in the mouse peritoneal cavity has been used as a measure of the ability of fibres to cause injury. The benefits of this model are that a well defined dose of respirable fibres can be introduced into an environment comprising macrophages and mesothelial cells and time courses followed; data obtained from this assay require careful interpretation but have shown clear effects of fibre length (Donaldson *et al.,* 1989) and dissolution (Donaldson *et al.,* 1994a).

PROLIFERATION

Brody and associates have described the proliferative events following inhalation exposure to chrysotile in a single high dose. There is increased turnover of epithelial cells, interstitial cells and cells in small blood vessels (e.g. Brody & Overby, 1989). The focal nature of this response is most striking, occurring at the terminal bronchiolar/alveolar junctions, where fibre deposition is very marked. It is not clear whether this response arises because of toxic effects of the fibres on cells or is due to the local release of growth factors. In addition, the relation of this single high dose exposure to a lower exposure is unknown. Increased proliferation in response to fibres which are known to cause fibrosis and cancer, two diseases associated with an overgrowth of cells, is a compelling idea.

Experiments at the Institute of Occupational Medicine (IOM), in the Colt Fibre Research Programme, with a glass fibre sample and an amosite asbestos sample of similar dimensions, given to rats by inhalation at equal fibre number, revealed increased proliferation with the amosite but not with the glass fibre (Donaldson *et al.,* 1994b). Rats exposed to these fibres did not develop pathological change with the glass fibre but significant numbers of tumours and severe fibrosis were found with the amosite asbestos. The ability to cause proliferation may therefore be an important property that defines a pathogenic fibre sample and the mechanism whereby proliferation is switched on is an important area of study. The glass and amosite samples alluded to above provide an ideal opportunity to examine the properties and biological activities that cause a fibre to be pathogenic.

A similar approach, modified to assess the mesothelial proliferation found after RIF exposure has been reported, showing an increased mesothelial proliferation

after short-term, high dose exposure to chrysotile (Coin *et al.,* 1991). However, instillation of a range of inflammatory insults that do not lead to mesothelioma, such as bleomycin and quartz, also produced increased mesothelial proliferation (Adamson *et al.,* 1994), which suggests that these effects may be non-specific. Monitoring the mesothelium for early markers of injury remains a desirable goal from the point of view of testing fibres as well as from the mechanistic viewpoint.

OXIDANT STRESS

The likely role of free radical/oxidant injury in the pathogenesis of fibre-related pathology has been addressed in the inhalation model by Mossman and associates. In this model the injury and subsequent inflammation and interstitial fibrosis caused in rats by 20 days exposure to 10mg/m^3 chrysotile was significantly decreased by treatment of the rats with polyethylene glycol-conjugated catalase, *via* implanted mini-pumps (Mossman *et al.,* 1990). Further work by this group has shown the induction of antioxidant enzymes in lungs of rats inhaling asbestos (Janssen *et al.,* 1992). This work points to a key role for free radicals in fibre related lung injury, which could emanate from the fibres or from the inflammatory leukocytes, and warrants further investigation.

IN VITRO MEASURES OF DIRECT DAMAGE CAUSED BY FIBRES

As might be anticipated it is much easier to detect damage to cells exposed to RIF in culture but Paracelsus' famous axiom - 'all substances are poisons, the poison resides in the dose' - may not always have been a consideration, particularly in the early work, when mg/ml doses of fibre were quite commonly used.

DEATH

There have been hundreds of papers documenting the ability of RIF (mostly asbestos) to kill cells and much was made in the early work of a potential relation between the ability of fibres to cause death of macrophages in culture and their fibrogenic potential. However, there has been a movement towards the idea that low doses of fibre occur in the lung during inhalation exposure and these are more likely to be associated with stimulation of harmful cell activities in contrast to the frank toxicity caused by higher doses *in vitro.* In addition there was a realisation

that cancer and fibrosis are *overgrowths* of tissue and so some failure of cell division control was involved. The strides made in understanding fibrosis and neoplasia in other fields allowed newer paradigms to become available and so the role of factors such as gene expression, cell stimulation and cytokine release increased in prominence in RIF research.

There does remain the concept of 'damage' to the normal tissue and loss of the normal architecture as factors leading to pathological change, particularly fibrosis. This 'damage' could arise from leukocyte products such as oxidants and proteases, as well as from direct effects of RIF.

FREE RADICALS AND RIF

The original model of Allison, Harington and associates (e.g. Harington *et al.,* 1975) invoked the plasma membrane as the focus of toxicity, as it is for quartz. Certainly asbestos, particularly chrysotile, in the absence of a protein coating directly damages membranes (Harington *et al.,* 1975). The ability of asbestos fibres to cause free-radical damage can be measured as lipid peroxidation and concomitant cell death (Goodlick *et al.,* 1989) which can be blocked by antioxidants, iron-chelating agents and hypoxia (Goodlick & Kane, 1986). In fact a unifying hypothesis has been suggested for the pathogenicity of all pneumoconiotic dusts, which centres around their ability to produce the hydroxyl radical at their surface (Kennedy *et al.,* 1989). This hypothesis certainly needs to be tested for a range of fibre types and some progress has been made on this (see below).

FREE RADICALS AT THE FIBRE SURFACE AND THE MODIFYING EFFECT OF THE TISSUE ENVIRONMENT

All of the studies demonstrating free radicals at the fibre surface have been carried out under protein-free conditions that never occur *in vivo*, where protein is ubiquitous in the lung lining fluid and in the interstitium. A coating of lung lining fluid on a fibre could greatly modify its surface free radical activity. The effect of a coating may not always be protective as shown by Lund *et al.* (1994) who demonstrated that the iron in asbestos bodies could be involved in free radical damage to DNA *via* Fenton chemistry. A programme of work on the ability of lung lining fluid, and residence in the lung in general, to modify the free radical activity of RIF is warranted so that the existing data can be evaluated and future

research planned. It is possible to detect free radicals associated with the fibre surface using electron spin resonance (ESR) (e.g. Fubini, 1993) but this is an expensive and difficult technique. It is also possible to detect free radicals by using supercoiled plasmid DNA (Lund & Aust, 1991) which is broken by free radicals. This assay has been used to examine the free radical burden associated with the surface of a range of RIF (Gilmour *et al.,*1994). Substantial differences were found between asbestos and a range of different man-made RlFs, with asbestos being generally more active than the man-made RlFs. In the case of asbestos, blocking studies with iron chelators and mannitol clearly implicate iron and hydroxyl radical in the fibre-mediated DNA strand breaks. Important questions for future research now relate to how these events are modified by proteins of the lung lining fluid, oxidants from phagocytes, such as H_2O_2, biologically available iron and iron binding proteins

GLUTATHIONE

The major non-enzymic antioxidant of the bronchoalveolar epithelium and lining fluid is glutathione (GSH) and together with its redox enzymes GSH forms a critical protective system in the lungs (Cantin & Begin, 1991). There is evidence that lowering the GSH levels in cells causes them to be more susceptible to oxidant injury of various types and this has been shown for mesothelial cells (Kinnula *et al.,* 1992) and for epithelial cells (Lannan *et al.,* 1994). Preliminary work suggests that decreasing the GSH levels in cells by pharmacological means renders them more susceptible to the harmful effects of fibres. Boehme *et al.* (1992) have reported decreases in GSH in alveolar macrophages treated with asbestos, probably due to export of the GSH from the cells and this could be important in modifying macrophage activity. Lombard-Gillooly and Hubbard (1993) have described more severe lung damage caused by quartz in mice that had their lung GSH levels decreased pharmacologically, than in GSH-sufficient mice. Dramatic decreases in epithelial cell GSH have been described in cells exposed acutely to cigarette smoke *in vitro* (Li *et al.,* 1994). Since many workers exposed to fibres smoke cigarettes, this could be a powerful factor enhancing toxicity to fibres. GSH homeostasis in fibre-exposed cells, and the modifying effect of concomitant exposures (e.g. cigarette smoke), has received very little research attention to date and is a target for future study.

MACROPHAGES, PHAGOCYTOSIS AND CLEARANCE

The alveolar macrophage is central to the clearance of fibres from the lung surface and failure to clear fibres may render them more likely to gain access to the interstitium. The volume at which a macrophage is 'overloaded' appears to be well defined for compact particles at 60% of the total volume of the cell (Oberdorster *et al.,* 1992), at which point phagocytosis is suppressed. Whether certain lengths or types of fibre have greater potential than others to induce suppression of phagocytosis is unknown. The ability of macrophages to migrate to the mucociliary escalator following phagocytosis is an important component of fibre clearance and it has been demonstrated that macrophages from asbestos-exposed lung have impaired ability to move (Donaldson *et al.,* 1990). Once again little is known of the mechanism whereby macrophage movement is inhibited or whether different fibre types differ in this property.

INTERACTION WITH MACROPHAGE RECEPTORS

Very little is known about the nature of the molecules on the surface of cells that are involved in interactions with fibres, apart from the best known opsonin IgG. It is well documented that coating fibres with IgG dramatically increases the ability of the fibres to up-regulate two events central to leukocyte-mediated damage to tissue in fibre-exposed lung, namely the oxidative burst (Scheule & Holian, 1989) and the release of the cytokine tumour necrosis factor (TNF; Donaldson *et al.,* 1992). It has recently been demonstrated that even the highly pathogenic long amosite fibres do not elicit any oxidative burst from rat alveolar macrophages unless they are first opsonised (Hill *et al.,* 1995). The lung lining fluid, in which the fibre first deposits, abounds with opsonins which increase during inflammation due to local secretion and plasma transudation. Although coating of the fibres with these proteins dramatically modifies their activity none of the hundreds of studies that have examined the ability of fibres to stimulate macrophage activities have systematically examined opsonisation. A systematic examination of the modifying effect of the lung lining fluid and its component opsonins (IgG, C3b and fibronectin) on the biological activity of RIF is therefore fundamental.

EPITHELIAL CELLS AND FIBROBLASTS

Despite a revolution in knowledge about the receptors that maintain the integrity of the lung by attaching cell to cell, cell to substratum and allowing leukocytes to

move from the vascular space to the interstitium and into the alveolar space (e.g. Albelda, 1991), little is known about the molecules that are involved in the interaction between fibres and the surface of epithelial cells. The central importance of this interaction makes this another important area for research. Evidence from studies with particulates suggests that it is interaction with the epithelium, facilitating the translocation of particles from the air space to the interstitium, that is a key defining event (Ferin *et al.,* 1990; Velan *et al.,* 1993). Chrysotile asbestos is highly pathogenic in rats and enters epithelial cells in the bronchoalveolar junctions where it can be seen in vacuoles and subsequently in the interstitium (Brody *et al.,* 1983); this is suggestive of active transport. This may not be the case for all fibres and clearly the factors that determine whether a fibre that deposits on an epithelium is susceptible to phagocytosis within a finite time, by the patrolling macrophages, could be central to determining if that fibre will be safely cleared or will enter the interstitium. It is possible to hypothesise that some fibre types, dependent on how they are opsonised, will interact more tightly, *via* receptors, with the epithelial cells and are destined for interstitialisation, whilst some bind more loosely and can be phagocytosed. The main luminal receptors in the bronchoalveolar epithelium are ICAM-1 and E-cadherin; ICAM-1 is inducible by inflammatory stimuli such as cytokines and oxidants (Albelda, 1991). Churg and co-workers (Keeling *et al.,* 1994) have also demonstrated that asbestos binds to epithelial cells by a mechanism that involves peroxidation and is enhanced by iron. Nothing is known of the specific receptor molecules that mediate the binding of RIF to epithelial cells and this represents a clear goal for future research.

The maintenance of a barrier between the interstitium and the alveolar space is an important function of the epithelium and increased epithelial permeability is found in asbestos workers (Gellert *et al.,* 1985). Recent work suggests that epithelial monolayers in culture are compromised by asbestos treatment *via* a subtle type of injury, as yet unelucidated, which increases their permeability (Peterson *et al.,* 1993); this injury was not characterised by cell death or changes in the normal actin cytoskeleton. Studies with an epithelial cell line (Donaldson *et al.,* 1993b) showed that long amosite, but not short amosite, could cause detachment of the epithelial cells in the absence of cell death. It is possible that long amosite can somehow down-regulate an RGD-binding receptor, which might be a fibronectin-binding integrin, and that this may lead to loss of attachment. Such an effect could be at the level of the integrin or the cytoskeleton and may be mediated by a free-radical type injury. Since epithelial leak and dysfunction could initiate and sustain inflammation, more data of the type described above are needed to fully elucidate the role of the epithelial injury in fibre-mediated lung disease.

An additional important factor is the role of the epithelium as a source of inflammatory mediators (e.g. Cohn *et al.,* 1993). No work, to date, has been published on the ability of fibres to stimulate release of mediators from these cells.

The epithelium also acts as a first line against inhaled organic xenobiotics and is equipped with enzyme systems to detoxify these, classically the cytochrome P450 family. These enzymes, although generally beneficial, can be harmful and convert polycyclic aromatic hydrocarbons to their proximate carcinogens. Another enzyme group, the Phase 2 enzymes (for example GSH transferases) is involved in further conjugation detoxification reactions. Since the P450 enzymes are inducible and up-regulation can potentially lead to a greater production of proximate carcinogens, any effect of fibres in up-regulating these enzymes could be important. This area is a particularly important one with regard to induction of P450 enzymes in smoking and other co-exposures or infection, and the modulation of GSH levels, and so requires research to clarify it.

Fibroblasts have been neglected as a target cell for direct effects of fibres. Given the likely importance of interstitialisation alluded to above, the interaction between RIF and fibroblasts, largely neglected up to this point, takes on more meaning and deserves to be addressed.

CHROMOSOME DAMAGE

There have been numerous studies showing that asbestos can cause chromosomal aberrations in various indicator cell lines as reviewed by Jaurand (1991). Human mesotheliomas have been found to have specific chromosomal losses and gains and Jaurand and co-workers (e.g. Yegles *et al.,* 1993) have studied chromosomal abnormalities caused in rat pleural mesothelial cells by asbestos and have reported metaphase and anaphase/telophase aberrations that are dose-dependent. In an attempt to relate these types of chromosome abnormalities to carcinogenesis Oshimura *et al.* (1984) compared this end-point with the ability to cause morphological transformation and found a good correlation. Future research in this area should investigate the ability of the non-asbestos RIFs to cause the same types of injury as asbestos, and the mechanism of the injury.

DNA DAMAGE

The release of free radicals from fibres in cells could, as result of a redox imbalance, cause free radical damage to DNA. Unscheduled DNA synthesis, suggesting increased DNA repair, has been described in rat pleural mesothelial cells exposed to asbestos but not attapulgite fibre (Renier *et al.,* 1990). No experiment has been carried out to determine whether or not this effect is a result of free radical damage. DNA strand breaks have been described in rat embryo cells treated with asbestos (Libbus *et al.,* 1989) and demonstrated by nick translation, both autoradiograpically and by scintillometry; the former precluded the complicating factor of DNA/fibre interaction with fibre during preparation. Interestingly this study included ground crocidolite and riebeckite which had little activity, but glass fibre had more activity than the crocidolite. The use of nick translation to detect DNA damage appears to be a sound one and should be used in a wider range of fibre types and lengths to determine its general relation to pathogenicity.

Leanderson has incubated 2-deoxyguanosine (Leanderson *et al.,* 1988) and calf thymus DNA (Leanderson & Tagesson, 1989) with various man-made fibres and assessed the formation of 8-hydroxydeoxyguanosine which results from the interaction of hydroxyl radical with guanosine. 8-Hydroxydeoxyguanosine was produced in each case with some synergistic effects with cigarette smoke. This system is less a model of what happens *in vivo*, since fibres are unlikely to make direct contact with DNA, but is more a non-specific measure of the ability of fibres to donate free radicals (as in the experiments of Gilmour *et al.* described above). However, the demonstration of products of oxidant damage to DNA following exposure of live cells to fibre would be very useful in exploring the role of free radicals in fibre-related disease.

APOPTOSIS

There is a clear link between oxidant stress and the induction of programmed cell death or apoptosis (Buttke & Sandstrom, 1994). Thus, if RIF deliver an oxidant stress, they may be able to induce apoptosis. This requires investigation, particularly if pro-apoptotic cytokines such as TNF are present in the micro environment, given the well documented ability of asbestos to stimulate TNF release from macrophages (Donaldson *et al.,* 1992)

THE PROBLEM OF SHORT-TERM ASSAYS THAT DO NOT TAKE ACCOUNT OF RESIDENCE IN THE LUNG

Virtually all of the assay systems described above for assessing cell damage take place over a very short time-scale; typically 24 or 48 hours. There is a general problem in using native fibres directly in short-term assays, when residence in the lung very likely changes their biological activity. This could occur almost immediately, on coating with proteins etc, or in the long-term by dissolution in the environment of the lung. One way around this problem would be to treat the fibres in ways that mimic residence in the lung prior to testing them (Donaldson, 1994); this would give a truer view of their likely biological activity in the lung in the long-term. It is, of course, difficult to choose an *in vitro* method to mimic the lung environment but a number of strategies are available for consideration (e.g. Donaldson *et al.,* 1994a).

VALIDATION OF ASSAY SYSTEMS AND GROWING DATABASE ON THE PATHOGENICITY OF RIF

There is a growing database of fibres whose pathogenic activity has been well characterised in well run long-term inhalation studies. The best known of these are the man-made vitreous fibres (MMVFs), refractory ceramic fibres (RCFs) and crocidolite of the Research and Consulting Company, Geneva (RCC) studies, the long and short amosite and the Code 100/475 vitreous fibres and silicon carbide used in the IOM Colt Programme studies. Samples of the original material from these studies should be procured and tested in assays of cell damage to validate the various assays of damage against pathogenicity.

RESEARCH NEEDS

An abbreviated list of priority areas is given in the summary below. Mechanism-based research on how RIF cause damage to cells and macromolecules has two interlinked aspects, namely risk assessment and an understanding of the disease process. Whilst activities in both areas seek the same goal, the immediate priorities may be different for the two. For the former the comparison of a range of man-made RIFs with asbestos and comparing these to their known pathogenicities is the focus; the role of chemistry/solubility and length are likely to be dominant. From the point of view of understanding the disease process, the continued testing of new hypotheses as to how fibres cause damage is the aim, using a small

number of fibre types. This second approach should produce the best possible assay system for risk assessment purposes at any given time and so both aspects should be encouraged.

SUMMARY OF PRIORITIES FOR RESEARCH ON THE DAMAGING EFFECTS OF FIBRES ON CELLS, MOLECULES AND DNA

In all of the following, the parameters of fibre length, fibre type/chemistry and durability need to be considered:

- the relation between direct and indirect (inflammatory cell-mediated) injury
- the significance of proliferation in RIF-exposed lung: its specificity to fibres; the mechanism of its induction; its importance in different compartments e.g. epithelium, mesothelium etc.
- oxidant stress in RIF-exposed lung; the induction of antioxidant enzymes and the transcriptional events that underlie them; the role of glutathione; the effect of cigarette smoking
- the production of free radicals at the surface of fibres and modifying factors: lung lining fluid; fibre coating; iron; leukocyte oxidant; dissolution
- the 'overload' volume in macrophages containing fibres of different lengths; fibre-mediated inhibition of macrophage movement
- fibre opsonisation and the lung lining fluid; the modifying effect of fibre coating in resting and inflamed lung in the long- and short-term
- epithelial cell adhesion molecules and the interaction with fibres; the relationship between adhesion of fibres to epithelial cells and phagocytosis/interstitialisation events
- role of inflammatory mediator release by fibre-exposed epithelial cells
- epithelial permeability following fibre exposure, its mechanism and significance

- effect of fibres on cytochrome P450 system in epithelial cells; effects of fibres on glutathione and Phase 2 reactions
- effects of fibres on fibroblast secretions and receptors
- validation of chromosome-damaging and DNA-damaging assays by using samples with known pathogenicity; assessment of the role of free radicals; new assays to detect DNA damage in RIF-exposed cells
- role of apoptosis in fibre-mediated cell death
- the damaging effects of fibres treated to mimic residence in the lung

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MECHANISMS OF DNA, MOLECULAR AND CELLULAR DAMAGE: ONCOGENES AND PROTOONCOGENES

S P Faux

CURRENT RESEARCH

Carcinogenesis is conventionally regarded as a series of events in a multistage process that has been divided into at least two stages, initiation and promotion. The initiation phase is believed to be the introduction of an heritable genetic change (mutation) resulting from carcinogen-induced DNA damage. Our current understanding of the initiation and promotional events leading to cancer reveals that the expression of oncogenes, activated by mutations, and tumour suppresser genes, which are inactivated by mutations, are important in the regulation of cellular function. Cell proliferation is strongly implicated in the process of clonal expansion of initiated cells and facilitation of other genetic alterations occurring during promotion and progression of tumours. Thus sustained cell proliferation appears to be a universal factor in the generation of human cancers by many cancer-inducing agents (Preston-Martin *et al.,* 1990). In these situations, cancer appears to result from genetic errors induced or fixed during the process of cell division, as increased mitogenesis increases the risk of multiple genetic defects including mutations, translocations and amplification of oncogenes. Both crocidolite and chrysotile types of asbestos cause chromosomal alterations in rodent and human mesothelial cells in culture (Lechner *et al.,* 1985), but bronchial epithelial cells seem to be more resistant to genotoxic and cytotoxic effects of asbestos (Lechner *et al.,* 1985; Mossman *et al.,* 1983). Accordingly asbestos seems to have more of a co-carcinogenic and promoter-like role in the development of lung cancer, whereas in the development of mesotheliomas, asbestos may be a complete carcinogen (Mossman *et al.,* 1990).

Searches for mutations in conventional oncogenes, such as *ras* and *myc*, that may have been activated as a result of asbestos-induced chromosomal changes have proved negative. One study by Vainio *et al.* (1993) found increases in K-*ras* oncogene mutations in lung tumours from asbestos workers. Many of these workers, however, were heavy smokers and on re-analysis of the data the authors suggested that the K-*ras* mutations were caused by smoking. In this situation asbestos would act as a promoting agent by conferring selective growth conditions for clonal expansion on these mutated cells.

There have been limited studies on the evaluation of oncogene products with monoclonal antibodies using immunoblotting techniques with human serum and tumours from individuals with an occupational history of asbestos exposure. In a study by Kishimoto (1991), there was no staining for K-*ras*, H-*ras* or *erb*-B in all eight cases of malignant mesotheliomas and in only one tumour was c-*abI* slightly positive. On the other hand, c-*myc* was positively stained in epithelioid type tumours and in the epithelioidal portion of biphasic type tumours. C-*neu*, c-*fos* and N-*myc* stained positive in almost all cases. Of these nine oncoproteins, c-*neu* was most frequently stained. These results may suggest that c-*myc*, N-*myc*, c-*neu* and c-*fos* oncogenes may play some role in the appearance of malignant mesothelioma related to asbestos exposure. In another study by Brandt-Rauf *et al.* (1992), levels of seven different oncoproteins were assayed with antibodies in 36 patients with asbestosis; at follow-up ten patients had developed lung cancer and two patients pleural mesotheliomas. Results for most of the individual oncoproteins were uninformative. However, the difference between cancer and non-cancer patients for the *ras* oncogene protein (also termed p21) was significant; seven of 15 asbestos-related malignancies (five lung; two pleural mesotheliomas) were positive (46.7%) *versus* two out of 28 non-cancer cases (7.1%; $p = 0.012$). In addition, six of the seven p21-positive cancer cases had positive serum samples prior to clinical diagnosis of the disease, suggesting that elevated serum p21 levels may be a useful marker for early detection in a significant percentage of respiratory malignancies. However, larger studies of this kind are needed to confirm the significance of these findings and also to discount the possibility that the rate of elevated p21 in the cancer cases compared with the non-cancer cases occurred by chance. The authors also did not examine the effect of smoking, even though the lung cancer cases were, or had been, smokers. This confounder needs to be taken into account.

Limited studies have also been performed on the evaluation of tumour suppressor genes and gene products in both mesotheliomas and lung carcinomas. Primary lung carcinomas often carry mutations in the p53 tumour suppressor gene. Most

of the mutations alter the conformation of the p53 protein into a more stable phenotype that makes it immunohistochemically detectable. In one investigation 70 primary lung carcinomas were examined for p53 protein accumulation with a polyclonal antihuman p53 antibody (Nuorva *et al.,* 1994). Abnormal accumulation of p53 protein was found in 36 tumours (51%), more often in patients exposed to asbestos than in patients without exposure (67% *versus* 40%, p=0.027). Significant association was also noticed between the accumulation of p53 and the asbestos content of lung tissue; 35% of the p53-positive patients had more than one asbestos body/cm^2 compared with 14% of p53-negative cases (p=0.046). Patients with strongly p53-positive tumours were heavier smokers (57.2 ±38.2 pack-years) than patients with p53-negative or lightly positive tumours (38.9 ± 19.9 pack-years; p=0.017). The findings of this study indicate that both asbestos exposure and heavy smoking can cause abnormal p53 accumulation suggestive of mutated p53. Mesotheliomas and nephroblastomas (Wilms' tumour) have many developmental, biochemical and histological similarities. Walker *et al.* (1994) have reported Wilms' tumour suppressor gene (WT-1) transcript expression in normal and transformed rat mesothelial cells. In addition, seven out of seven human mesothelioma cell lines also expressed the WT-1 transcripts (Walker *et al.*, 1994). Recent investigations by Amin *et al.* (1995) have shown expression of WT-1 mRNA in 16 out of 19 mesothelioma cell lines and five out of eight malignant mesothelioma tumours. In contrast, WT-1 mRNA was not detected in non-small cell lung cancer lines or carcinomas. The detection of WT-1 mRNA or protein may thus provide a specific molecular or immunohistochemical marker for the differentiation of mesotheliomas from other pleural tumours (Amin *et al.,* 1995: Walker *et al.,* 1994)

Traditional approaches for identifying asbestos-induced oncogenes by rodent transformation assays have been inconclusive. For example, transfection of murine NIH/3T3 cells with DNA from a human mesothelioma showed that the transforming DNA was not homologous to any of the *ras* oncogene family, and the identification of the gene(s) remains unsolved (Barrett *et al.,* 1989). However, in another study, analysis of asbestos-induced Syrian hamster tumour cell lines showed that activated H-*ras* oncogenes were present in approximately 50% of the tumour-derived cell lines while the non-tumourigenic immortalised cell lines lacked the H-*ras* oncogene (Barrett *et al.,* 1989). Other groups have examined normal human mesothelial cells and mesotheliomas to discern differences in expression of oncogenes encoding growth factors (Gerwin *et al.,* 1987; Versnel *et al.,* 1988). These studies show increased c-*sis*, an oncogene encoding the *beta* chain platelet derived growth factor (PDGF), in mesotheliomas. Thus PDGF, a documented mitogen, may be important in conferring autonomous tumour growth.

To date no information is available on man-made mineral fibres (MMMF) with respect to oncogenic mutations or expression of oncogene proteins.

In recent years, important advances have been made towards understanding the molecular mechanisms of asbestos-induced carcinogenesis and there is sufficient evidence to suggest that the cytotoxic, genotoxic and proliferative effects of asbestos fibres are mediated in part by active oxygen species (AOS) that are produced by both cellular and acellular pathways (Mossman & Marsh, 1989). There is convincing evidence that prolonged oxidative stress, that is chronic exposure to AOS in the form of singlet oxygen, superoxide radicals, H_2O_2 and/or hydroxyl radicals can result in cell injury and play a role in several stages of carcinogenesis (Cerutti, 1985). Molecular epidemiological studies indicate that mutagenic activation of protooncogenes and inactivation of tumour suppresser genes can lead to 'response modifications' of target cells required for tumour promotion and progression (Cerutti, 1988). Unlike the more permanent genetic changes, modulation of growth and differentiation occurs in a large proportion of cells of a tissue exposed to oxidative stress and involves reprogramming the expression of entire families of genes. Importantly, AOS induce transcription of the immediate early genes, such as c-*fos*, c-*jun* and c-*myc* (Crawford *et al.,* 1988) that are important in cell proliferation. Experimental models have revealed that alveolar macrophages (AMs) accumulate at sites of fibre deposition, an event preceding inflammation and histopathologic indication of disease. Infiltration of AMs is followed by a generalised inflammatory recruitment response characterised by elevated cell numbers and neutrophils in bronchoalveolar lavage. These cell types and macrophages may be important in lung defence or causally related to lung disease in producing cytokines, chemotactic factors and AOS. The asbestos or AOS may act as chemotactic factors for the prolonged inflammatory responses that occur after exposure to pathogenic asbestos but are less pronounced after inhalation of MMMF. AOS also appear to be released from cells after interaction of asbestos or non-asbestos fibres with the cell membrane or after frustrated or incomplete phagocytosis. For example, increased amounts of superoxide radicals were produced after hamster and rat AM were exposed *in vitro* to long thin fibres, whereas generation was minimal after exposure of cells to shorter or non-fibrous materials (Hansen & Mossman, 1987). Thus, the dimensional properties of fibres may influence the manner in which they are phagocytosed by cells with consequent release of AOS. Durability has also been shown to be important during the promotion and progression stages of asbestos-induced tumours and may also result in continued release of AOS. AOS may also be produced from iron on the surface of fibres, where asbestos has been shown to produce many of the same biochemical reactions as iron in the generation of the

reactive metabolites of oxygen. These toxic reactions produced by asbestos *in vitro* can be ameliorated in the presence of iron chelators. However, the second mechanism of AOS species production would not be applicable in fibres containing little or no iron. The reason for the inclusion of this section will become clear in the following paragraphs.

Recent advances in molecular biology now allow examination, using gene probes, of isolated events occurring during fibrosis and carcinogenesis and specific alterations in gene expression at certain stages of the carcinogenic process, including cell proliferation. This approach has recently been adopted by Mossman and co-workers with asbestos and other non-asbestos fibrous materials. If asbestos acts as a promoter of cell proliferation, it should increase the expression of genes important in cell proliferation, such as c-*fos* and c-*jun*. Studies by Heintz *et al.* (1993), examined the expression of c-*fos* and c-*jun* mRNA in confluent cultures of rat pleural mesothelial (RPM) and hamster tracheal epithelial (HTE) cells treated with sublethal concentrations of asbestos that induced cell proliferation in both cell types. In response to TPA, a classical tumour promoter, both RPM and HTE cells displayed a transient increase in c-*fos* and c-*jun* mRNA that peaked at one hour and subsequently decreased. This phenomenon is a hallmark of the early response gene pathway. In contrast, the patterns of gene expression were different in RPM and HTE cells after asbestos exposure. Crocidolite and chrysotile asbestos induced a dramatic increase in c-*fos* mRNA in RPM cells, but c-*fos* was not induced in HTE cells. This result shows that the regulation of c-*fos* expression varies in different cell types. In both HTE and RPM cells, asbestos induced increases in c-*jun* mRNA that persisted at high levels, nearly five-fold greater than control cells, for at least 24 hours. This persistent induction of c-*jun* and/or c-*fos* is significant, as the induction of transcription by activator protein-1 (AP-1) binding proteins requires sustained expression of these factors. In these studies the authors observed increased AP-1 DNA binding with respect to controls following asbestos treatment, which is consistent with the mRNA Northern blot data.

The induction of gene expression in RPM cells is dose-dependent and was not observed with polystyrene beads or rieberkite (chemically similar to crocidolite), which are non-fibrous and non-carcinogenic particles. These data suggest that fibre geometry is a critical determinant for the induction of the protooncogenes and that the response was not stimulated by the presence of a solid body alone. These results may suggest a model for the induction of neoplastic disease by asbestos. The protooncogenes c-*fos* and c-*jun* encode a family of AP-1 transcription factors that form homodimeric and heterodimeric protein

complexes. These AP-1 transcription factors bind to specific regulatory sequences in DNA and control the transition from G1 to S phase in the cell cycle with subsequent cell proliferation.

In the latest studies from Brooke Mossman's laboratory, erionite, a fibre extremely potent in inducing mesotheliomas in man and rodents, caused more striking increases in mRNA expression of c-*fos* and c-*jun* in RPM cells than did crocidolite asbestos. In further studies, man-made vitreous fibres (MMVF-10) and refractory ceramic fibre-1 (RCF-1) both elevated c-*fos* and c-*jun* protooncogene expression in the two target cells of asbestos-induced disease, that is the pleural mesothelial cell and tracheal epithelial cell (Janssen *et al.,* 1994). However, in comparison with asbestos, protooncogene induction was less pronounced and occurred only at higher concentrations. These fibre types have also been shown to cause the development of tumours after intrapleural or intratracheal injection techniques, but only RCF has been shown to cause pulmonary tumours and mesotheliomas in rats following inhalation; this may be a consequence of increased durability in the lung or pleura. These results suggest that MMVF-10 and RCF-1 (as well as a number of other fibre types) may have the capacity to cause tumours if present at the target site in sufficient concentration.

As previously stated, AOS have been implicated in asbestos toxicity. Studies by Janssen *et al.* (1994) examined whether AOS, in the form of H_2O_2, play a role in the induction of c-*fos* and c-*jun* in different target cells of asbestos-induced disease. The results showed that exposure of HTE cells to H_2O_2 for two hours caused a dramatic induction of c-*fos* and c-*jun,* which decreased to control levels at 24 hours. Interestingly, no mRNA levels of c-*fos* were observed in HTE cells after exposure to asbestos, indicating that AOS production by asbestos may be insufficient to induce c-*fos* in these cells. Other experiments also indicate that c-*fos* and c-*jun* are not induced in RPM cells following exposure to H_2O_2 or other AOS generating systems. However, under the same conditions, gene expression of manganese containing superoxide dismutase and haeme oxygenase (enzymes induced by oxidants) is elevated, indicating an oxidative stress response. These results indicate that the pathways of c-*fos* and c-*jun* activation triggered by asbestos and AOS in HTE and RPM cells are different. More studies are required to elucidate the mechanism of c-*fos* and c-*jun* activation in these cells.

A recent inhalation study in rats revealed increases in c-*jun* in mRNA in whole lung after 20 days of crocidolite exposure, with no increase at earlier time points (Quinlan *et al.,* 1994). A similar result has now been reported following probing of Northern blots with c-*fos* cDNA.

Currently, the studies described above provide some of the molecular understanding of the consequences of asbestos exposure that may cause cancer. These molecular markers of disease used in appropriate *in vitro* and *in vivo* models should help to predict the carcinogenic and fibrogenic potential of asbestos and non-asbestos fibres.

RESEARCH NEEDS

Future studies are required to elucidate the mechanism(s) of c-*fos*/c-*jun* expression in these cell types by asbestos. The involvement of AOS needs further study. There is also much evidence to implicate oxygen radicals and elevated cytosolic calcium concentrations in cell toxicity *in vitro*. Recent studies have shown the involvement of intracellular calcium in crocidolite-induced DNA strand breakage (Faux *et al.,* 1994). Crocidolite-induced oxygen radicals were shown in this study to induce DNA strand breakage through calcium by disturbing intracellular calcium homeostasis. Strand breaks in DNA are known to be amplified by endonucleases causing a distortion of chromatin structure. It is possible that this conformational change may induce the protooncogenes in the absence of other signals. Glutathione (GSH) plays a unique role in the cellular defence against AOS and cell toxicity by oxidative stress is often preceded by depletion of intracellular GSH. Both calcium and GSH need to be assessed in relation to activation of c-*fos*/c-*jun*. Little is known about the mechanisms of cellular signalling which trigger responses to asbestos and non-asbestos fibres. However, the mechanisms of cell signalling by crocidolite asbestos are similar to those observed with TPA and appear to be mediated by protein kinase C (Perderiset *et al.,* 1991). In conclusion, multiple mechanisms may exist for the induction of the early response gene pathway by asbestos and other fibres.

The above approach to looking for protooncogenes that are involved in cell proliferation is a big step forward in assessing fibre-induced toxicity and potential carcinogenicity if used in the appropriate *in vitro* model, e.g. pleural mesothelial and tracheal epithelial cells. If a novel fibre is able to induce c-*fos*/c-*jun* in these *in vitro* models then the next strategy would be to look *in vivo* in the most appropriate inhalation model.

The protooncogenes c-*fos* and c-*jun* may not be the only genes that are involved in asbestos-induced carcinogenesis as other potential candidates have not been looked for. A technique of differential mRNA display is a novel method to detect all mRNA species that are expressed in a particular cell. This technique therefore

allows investigators to compare multiple mRNA populations derived from control and treated cells and allows qualitative and quantitative differences in expression of mRNA to be measured. Differential mRNA display may be a step forward in the identification of such candidates.

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MECHANISMS OF DNA, MOLECULAR AND CELLULAR DAMAGE: CYTOKINE EXPRESSION IN LUNG MACROPHAGE POPULATIONS

C Meredith

INTRODUCTION

Knowledge concerning mineral fibre induced disease in the lung has increased over the last decade due to intense research activity in the area. Based on expert clinical observations, extensive epidemiological studies and the use of experimental systems, there is now clear evidence to indicate that the development of fibrosis or carcinogenesis is related to differences in overall dimensions, chemical composition and durability of the fibre in lung tissue. However, the precise cellular and molecular events which lead to the observed pathological changes remain to be elucidated. One of the most important areas that is now being investigated is the effect of man-made mineral fibres (MMMF) on the development of pulmonary pathology. Few human data are available to assess the effects of MMMF and research efforts so far have focused on the use of selected experimental models to understand or to predict the effects of MMMF on lung pathology. As knowledge of the regulation of cellular activity at a molecular level has increased it has become clear that both the normal and abnormal pathology of the lung are significantly influenced by subtle alterations in the regulatory cytokine network. This paper summarises current concepts regarding cytokine expression in lung cell populations and describes current understanding of the effects of MMMF on cytokine expression. The work seeks both to understand the molecular mechanisms associated with MMMF induced fibrosis and carcinogenesis and to devise appropriate short-term predictive tests.

CYTOKINES OF THE LUNG

Cytokines are extracellular signalling proteins secreted by specific effector cells, their prime function is to modulate the behaviour of closely adjacent cells although more distant effects are also documented. Cytokines can influence proliferation (as growth promoters or inhibitors), motility, cytoskeletal arrangement, contractility and the production of extracellular matrix proteins. Cytokines secreted by one cell type can often influence cytokine expression in the target cell thus establishing complex networks of regulation. Originally cytokines were thought to be associated primarily with regulation of the immune system (classified as monokines and lymphokines); the situation is now known to be much more complex and cytokine expression in the lung is a reflection not only of immunological activity but also of proliferative and growth responses. It is not always possible to ascribe a particular activity to a single cytokine, for the effector networks are often diverse and complex. However, patterns of cytokine expressions are now being established for several organ systems, including the lung, and it is clear that modulation of cytokine expression can be indicative of abnormal responses within tissues. Whilst it is premature to link up-regulation or down-regulation of any given cytokine with a specific pathological condition, there exists considerable potential to analyse patterns of cytokine expression in the early stages of the disease process, to identify possible markers and to postulate mechanisms *via* which the pathological condition is induced.

Cytokines that are known to be expressed in lung tissue (either by lung macrophages or by constituent cells) include platelet derived growth factor (PDGF), fibroblast growth factor (FGF), interleukin 1 (IL-1), tumour necrosis factor-α (TNFα), macrophage inflammatory protein-1 (MIP-1), transforming growth factor-β (TGFβ), epidermal growth factor (EGF), interleukin 6 (IL-6) and insulin-like growth factors (IGF). The network of effects that are induced by these cytokines in lung tissue have been comprehensively reviewed by Kelley (1990).

Most of the effects of these cytokines are mediated *via* interaction with a specific cytokine receptor on the cell surface of the target cell. It follows, therefore, that regulation of cytokine receptor expression could also be an important contributory event to homeostatic or pathologic pulmonary function. Cytokine receptors which have been identified in lung tissue include those for IL-1, TNF, IL-4, IL-6, IL-8 and interferon-γ (IFNγ). The regulation of cytokine receptor expression in the lung has been reviewed briefly by Shepherd (1991).

CYTOKINES IN LUNG DISEASE

Recent advances in cellular and molecular biology have led to a better understanding of the interactions between cytokines in the development of lung disease. It has been demonstrated that alveolar macrophages can produce both IL-1 and TNFα which can stimulate fibroblast proliferation, whereas IL-1 or IFNγ (from T-lymphocytes) can synergise with TNFα to inhibit fibroblast proliferation, depending on the state of activation of the target cell (Elias *et al.,* 1990). These studies also showed that fibroblasts themselves could produce the cytokines IL-1α, IL-1β and IL-6 which would augment local inflammatory events.

Direct evidence for the involvement of TNFα comes from the murine model of Piguet *et al.* (1990) who demonstrated that TNFα was expressed in the lung during the development of silica-induced pulmonary fibrosis and that the effects could be inhibited by pretreatment with an anti-TNFα antibody. This result directly implicates the cytokine TNFα in the fibrogenic pathway. The same group had previously demonstrated the role of TNFα in bleomycin-induced pneumopathy and fibrosis (Piguet *et al.,* 1989). It has been shown that human alveolar macrophages exposed to asbestos both *in vitro* and *in vivo* can express a range of cytokines and prostaglandins (Perkins *et al.,* 1993). *In vitro* experiments demonstrated that TNFα was the major cytokine released by asbestos exposed macrophages whereas IL-1β, TNFα and IL-6 were all enhanced in cells from asbestos-exposed patients.

CYTOKINES AND MMMF

In studies conducted at BIBRA Toxicology International over the past three years (funded by the Health and Safety Executive), an attempt has been made to use molecular biological techniques, including cytokine expression analysis, to screen MMMF for their pathogenic potential. *In vitro* studies using cultures of alveolar or pleural macrophages from C3H mice showed that exposure to MMMF induced a small but reproducible rise in cytokine gene expression particularly IL-1β mRNA (Hudspith *et al.,* 1994). However when cells were activated with lipopolysaccharide, exposure to MMMF partially inhibited the expression of cytokines including IL-1β and TNFα. This was not due to any cytotoxic effect and appeared to be independent of the type of fibre employed, including refractory ceramic fibre (RCF)1, RCF2 and glass fibres.

In parallel *in vivo* studies, using lung macrophages from mice exposed by inhalation to MMMF for two hours per day for two weeks, it was shown that freshly isolated alveolar cells produced no IL-1β nor TNFα mRNA in either control or MMMF exposed animals. There were interesting effects in the pleural cell populations; in particular it was demonstrated that RCF1 increased the levels of TNFα mRNA both during the exposure period and during a recovery phase. RCF2 had no such effect, whereas glass fibre produced only a mild transient elevation in pleural macrophage TNFα mRNA. (Hudspith *et al.,* 1993).

These recent data are particularly interesting since they suggest that whereas *in vitro* exposure to MMMF gives similar cytokine responses, *in vivo* exposure results in cytokine modulation *ex vivo* which is fibre specific. Additionally the absence of observable effects in alveolar macrophage cultures contrasts with the clear evidence of increased TNFα expression in pleural macrophage cultures following MMMF exposure. This does not necessarily indicate that these are the changes that occur in the whole animal; for that to be deduced it would be necessary to conduct *in situ* hybridisation analysis on tissue from fibre-exposed animals. Additionally, these observations only reflect transcriptional events, and analysis of secreted cytokines using ELISA or Western blot techniques would be necessary to confirm the modulation of the effective extracellular concentration of these cytokines. Nevertheless, the technology offers great potential for the rapid screening of macrophage cultures obtained from fibre-exposed animals for the dysregulation of a wide range of cytokines which might be implicated in fibre-induced lung disease.

There are two tentative conclusions which may be drawn from this work. Firstly, it appears that such a short-term inhalation exposure may offer the possibility of screening fibres for pathogenic potential. Secondly, the finding that activation of pleural macrophage cytokines occurs in the absence of alveolar macrophage activation suggests a primary interaction of MMMF with yet another lung cell type.

RESEARCH NEEDS

The research described in this brief paper has been performed using a murine model. This was necessary because at the time the project was conceived, the only molecular probes available were for murine cytokines. It would now be appropriate to transfer this model into a species such as the rat which is more routinely used in toxicity studies and for which molecular probes are now becoming available in this and in other laboratories.

The research described here concentrates mainly on modulation of expression of the cytokines from the IL-1 or TNF family; clearly there are a number of other cytokines involved in inflammatory and fibrogenic pathways. Future research should focus on the elucidation of these pathways using molecular and immunochemical techniques. Additionally, research efforts should concentrate on *in situ* hybridisation analysis of lung tissue from animals exposed to fibres in order to confirm that cytokine modulation seen in the *ex vivo* analysis reflects the events which occur *in vivo*.

The observation that pleural cell macrophage activation can occur without prior activation of alveolar macrophages suggests that other lung cell types may be involved. Future research should focus on this possibility using both *in vitro* techniques and short-term inhalation exposures.

No dose-response data are yet available for these documented effects of MMMF on cytokine expression. Studies should be established to gather data on dose-response effects using carefully monitored inhalation exposure with appropriate dosimetry measurements.

Experiments in progress at BIBRA are assessing the effects of MMMF exposure on protooncogene expression in whole lung and preliminary data have been reported recently (Hudspith *et al.,* 1995). These experiments also use molecular techniques to monitor specific protooncogene expression and form a natural extension to the cytokine experiments, focusing on the control of proliferation in lung tissue. This type of research should be carried forward and could be performed as adjunct studies to major short-term or long-term inhalation exposures.

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THE PHYSICAL AND CHEMICAL PROPERTIES OF FIBRES

J Young

THE FIBROUS MATERIALS

The extensive and continually developing range of technologically significant natural asbestiform fibres (Rendall, 1991), natural non-asbestiform fibres (Gibbs, 1991) and man-made mineral fibres (Young, 1991) presents a complexity of fibre-product forms, chemical compositions, crystalline and non-crystalline microstructures, surface treatments and size distributions. Subsequent storage and use of fibres can significantly modify their bulk and surface properties and inhalation can result in coating with body fluid, hence adding to this complexity.

For example, the term 'aluminosilicate refractory ceramic fibre' represents a family of products of varying silica, alumina and additional oxide compositions that, in the 'as-manufactured' state, are normally amorphous and smooth-surfaced (Young, 1991; Glass *et al.,* 1995). When used in clean atmospheres at high temperatures the fibres undergo devitrification, with associated chemical and physical changes to their bulk and surface properties (Brown *et al.,* 1992; Laskowski *et al.,* 1994). These changes are often significantly different when high temperature exposure takes place in an industrial environment, for example in an annealing furnace or pottery kiln (Dietrichs *et al.,* 1986). Further, the storage of fibres in damp environments can result in preferential leaching and chemical modification to fibre surfaces. Fibre inhalation results in coating with lung lining fluid and therefore also modifies the surface (Lund & Aust, 1991).

The complexity of this wide and diverse range of inorganic fibrous materials comprising 'as-manufactured' fibres and fibres modified by storage and use suggests that fibrous materials should be classified and studied in terms of key parameters associated with their chemical and physical properties such as their

bulk and surface chemistry and microstructure, surface charge, fibre diameter, etc, rather than as families of fibrous material, such as ceramic fibres or glass fibres.

In general, knowledge of the effects of use on the bulk and surface properties of fibres is limited to specific materials and applications. There is therefore a need for research to characterise the major effects on fibres of storage and use and to allow such fibrous materials to be classified and understood within the materials framework identified above.

THE HEALTH HAZARD

Faced with this complexity of potentially respirable fibrous materials, an approach is required which would provide an initial and reliable screening of the potential of a particular fibrous material to cause disease. Ideally, this would involve measurement of the physical and chemical properties of the particular fibre and an assessment based on comparing these measurements with established relations between these properties and the likelihood of disease initiation, and also the use of an accredited *in vitro* screening test. The results of this initial screening might then necessitate further *in vitro* and/or *in vivo* testing to confirm a potential hazard, but the aim should be to reduce the need for expensive animal testing for each and every 'as-manufactured' fibrous material or subsequent application.

In terms of establishing an accredited *in vitro* screening test, a number of authors including Scholze and Conradt (1987) have developed solubility tests based on the controlled exposure of fibres to simulated extracellular fluids and this type of testing has also been pursued by fibre manufacturers (Jubb & Martin, 1993). While such a solubility test would be ideal in cost terms and could, if necessary, be followed by appropriate *in vitro* cell testing, the validity of *in vitro* solubility testing has been questioned by Morgan and Holmes (1986) and remains a matter of conjecture.

The need for an assessment based on comparing measured fibre properties with established relations between fibre properties and disease potential was probably first expressed by Timbrell (1982) and has been well articulated by Spurny (1991). The latter proposes an 'empirical fibre toxicity model' and an associated strategy for toxicity testing. The proposed model aims to predict carcinogenic potential and identifies factors such as fibre size, fibre persistency and zeta potential as important contributors to a functional predictive equation. The testing strategy

moves from an initial screening using this proposed model through various levels of biological testing.

The development of such an 'empirical fibre toxicity model' demands identification of both those physical and chemical properties of a fibre which are significant in assessing the potential health hazards posed by a fibrous material and the relation between these variables or attributes and the potential health hazard. This information derives primarily from *in vitro* and *in vivo* studies. From the perspective of experimental design, careful consideration of the choice of biological test systems and fibre samples is required. The complexity of fibre properties necessitates the application of statistical experimental design methods to identify those that are significant (Montgomery, 1991).

The storage and use of fibres can significantly modify their physical and chemical properties as discussed above, and these effects, together with inhalation with other airborne pollutants or cigarette smoke, or inhalation of fibres with surface-absorbed organics, may affect the potential health hazard (Glass *et al.,* 1995). These factors also require inclusion in any empirical model.

TEST FIBRES AND THEIR CHARACTERISTICS

Standard test fibres with well-characterised and accredited properties are required for *in vivo* and *in vitro* mechanistic studies to enable the effects of specific fibre characteristics to be separately identified. For example, if the key question is the effect of fibre dimensions then an appropriate series of fibres is required with accurately defined dimensions; all fibres would be manufactured from the same, likely very durable, fibrous material. The physical and chemical properties of the fibres would need to be characterised before and after testing, and the measurements would ideally be accredited as traceable to a reference standard using, for example, the National Measurement Accreditation Scheme (NAMAS). Similarly, fibres of varying surface chemistry but common fixed dimensions would be required to assess chemical effects.

The subsequent application of an 'empirical fibre toxicity model' to assess the hazard posed by a particular fibrous material also would require the ability to characterise accurately the physical and chemical properties of the fibrous material concerned. This process would also necessitate the preparation of a truly representative sample of that fibre.

RESEARCH ISSUES

The development of a well-founded and broadly applicable 'empirical fibre toxicity model' provides an appropriate target for research endeavours.

The following research issues arise specifically in relation to the fibrous materials:

There is a need to characterise, study and classify fibres and fibrous materials in terms of their physical and chemical properties, for example their bulk and surface chemical composition, bulk and surface microstructure, surface charge, fibre diameter, etc, rather than in terms of the 'families' of fibrous materials to which they belong. This will engender a more scientific and more broadly applicable approach.

Considering the total range of 'as-manufactured' fibrous materials, the potential effects of fibre storage and fibre use, and therefore the large and complex potential range of distinct inhalable fibrous materials, there is clearly a need to prioritise appropriately the fibres that should be studied.

With a few specific exceptions, the effects of fibre storage and use on the physical and chemical properties of fibres are little understood and research is needed to address this shortcoming.

The question of whether the coating of inhaled fibres by lung fluids undermines the dependence of their toxicity on the surface properties of the fibres, and the question of the effect on fibre toxicity of concomitant inhalation of other airborne pollutants should be addressed.

There is a need to identify experimentally those aspects of a fibre's physical and chemical properties that determine the health hazard posed by inhalation. This determination requires good experimental design including the selection and preparation of standard fibre samples for biological studies, and appropriate characterisation before and after testing.

A range of standard fibre specimens providing an appropriate range of physical and chemical properties has therefore to be agreed by the fibres research community and manufactured, characterised and accredited for experimental use. An appropriate approach would be to set up a bank of

standard fibres to be used by the research community that would then enable comparison and pooling of research results. The methods employed for physical, chemical and biological characterisation should be agreed and the use of NAMAS-accredited methods and measurements would enable traceability. It has also been suggested by Dr Donaldson that samples should be returned to the bank following their use in experiments so that further testing may be undertaken which would build on the history of previous testing environments. For example, an *in vivo*-tested fibre with a known history could be further tested *in vitro*.

RESEARCH NEEDS

The necessity of providing a bank of standard test fibres requires a significant level of collaboration, ideally between researchers and industry, for its delivery. In particular, there is a need for:

the fibres research community to specify the range and quantities of test fibres required,

the manufacture of standard fibres, which will demand use of a range of plant currently available in industry, research agencies and universities,

NAMAS-accredited physical, chemical and biological characterisation, which will require facilities in different locations,

agreement concerning the location and administration of a bank.

To characterise the effects of use on fibres, good collaboration is required between researchers and users, in particular industrial users, to enable realistic samples to be identified and studied.

Overall, it is clear that the achievement of good research into the health hazards posed by fibrous materials requires good interdisciplinary programmes of work involving industry, government agencies and universities and involving a spectrum of expertise that spans engineering, materials science, physics, chemistry and biomedical science.

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BODY DISTRIBUTION AND BETWEEN SPECIES DOSIMETRY

P M Hext, C G Collier and D B Warheit

INTRODUCTION

Distribution and dosimetry of fibres in the body depends upon their deposition and clearance in the respective region, which may ultimately influence the initiation and progress of fibre-induced disease. This paper reviews briefly the behaviour of fibres following administration by inhalation to the respiratory tract and injection into the peritoneal cavity. Most of the research has been conducted using the rat as the animal model and therefore there is a paucity of data on different species for most aspects studied.

THE RESPIRATORY TRACT

DEPOSITION

General patterns of deposition of non-fibrous particles in the respiratory tract in the human, hamster, mouse, rat, guinea pig, dog and monkey have been reviewed by Schlesinger (1985) and are considered relevant to fibres in general terms when compared on the basis of aerodynamic size. However, deposition due to interception will enhance overall deposition efficiencies for fibres.

Deposition is influenced by the structure of the respiratory tract. Assuming nasal breathing, the complex structure of the upper respiratory tract (nasopharyngeal region) in the rat and other commonly used experimental species means that the majority of particles greater than approximately 6μm aerodynamic diameter and an appreciable proportion of smaller particles will deposit in this region.

The anatomy of the human lung differs from that of experimental animal species. Humans display symmetrical dichotomous airway branching patterns which favour deposition at the bifurcations. Rodents show highly asymmetric monopodal branching, which should in theory reduce the tendency for concentrated areas of deposition. In addition the distal airways of the human lung are fundamentally different from those of rodents because they contain several generations of non-respiratory bronchioles and three generations each of respiratory bronchioles and alveolar ducts. In guinea pigs and hamsters these respiratory bronchioles are poorly developed and in rats and mice are absent. As a consequence, respirable fibres tend to deposit on alveolar duct bifurcations in rodents (Brody & Roe, 1983), whereas in humans they concentrate on the final respiratory duct bifurcation. Another important potential physiologic difference stems from the fact that rodents are obligate nose breathers whereas humans may favour oral breathing while speaking, or during exercise, thus permitting enhanced particle penetration to the lungs (Lippman, 1977).

The above anatomical and related airflow differences between rodents and humans often result in marked inter-species differences in deposition efficiencies (see Schlesinger, 1985). The maximum physical diameter for fibres depositing in the human lung is about 3.5μm (Timbrell, 1965) whereas that for rats it is lower, about 2μm (Morgan *et al.,* 1980).

Mathematical modelling of deposition has been conducted and qualitative aspects of fibre and particle deposition are well understood. Practical measurements of deposition in animals and the mathematical modelling of deposition in man (Asgharian & Yu 1988; Balashazy *et al.,* 1990) and experimental animals (Asgharian & Yu 1989; Yu *et al.,* 1994) show good agreement.

An alternative route of administration used to study the behaviour and effects of fibres in the respiratory tract is intratracheal instillation. Pritchard *et al.* (1985) demonstrated differences, as expected, in distribution of radioactive dust in the lung following administration by this route as compared with inhalation and this is considered applicable to fibres also. While instillation as a route of administration is of limited relevance to man, it can provide useful information on fibre fate and dissolution and mechanisms of action within lung tissues. In addition, if limited quantities only of a specific material are available, intratracheal instillation would be the route of choice.

CLEARANCE

Three pathways exist for clearance of material from the site of deposition - mechanical, translocation and dissolution. For non-fibrous particulates, the mechanisms have been extensively reviewed by Oberdorster (1988) and in general they are appropriate to fibres. However, differential clearance attributable to different fibre lengths is known to occur (Abraham *et al.,* 1988), resulting in a more rapid clearance of short fibres and hence an increasing proportion of longer fibres within the lung with time. As a consequence, nominal short fibre preparations may lead to retention of long fibres in the lung.

Mechanical clearance occurs *via* the mucociliary escalator or nasal mucus flow to the throat followed by expectoration or swallowing. For material deposited in the alveolus, this is principally macrophage mediated. This clearance mechanism is believed generally to be independent of the material, provided the normal clearance mechanisms are unimpaired. Many studies have shown that alveolar mechanical clearance rates vary markedly between species and even between strains, with humans showing rates ten-fold lower than rats, mice and guinea-pigs (Oberdorster, 1988). Macrophage mediated clearance is recognised as being responsible for the clearance of fibres shorter than 10μm (Morgan *et al.,* 1982). Fibres longer than 10μm (possibly 20μm) cannot be phagocytosed by a single macrophage and do not clear from the lung by this route (Timbrell & Skidmore, 1971).

The clearance of insoluble particles from the conducting airways mediated by mucociliary activity is rapid and is usually complete in 24-48 hrs, but it may take longer (Gore & Patrick, 1982). It is now believed that instead of the mucous lining layer of the respiratory tract acting as a barrier between deposited particles and epithelial cells, lining fluid-particle interactions may favour particle/cell interactions due to surface tension effects (Gehr *et al.,* 1993). Long-term retention of a small fraction of fibres in the trachea has been observed by Morgan *et al.* (1993) following intratracheal instillation.

Translocation of fibres to the interstitium, lymphatics or pleura is known to occur and is dependent on fibre size, both length and diameter. Morgan *et al.* (1977) demonstrated gross translocation of fibres in the lung using radioactive fibres. Translocation into the interstitium has been described following short- or long-term exposure of rats to fibres (Pinkerton *et al.,* 1984; Chang *et al.,* 1988). Similar findings have been described in asbestos workers (Churg & Wright, 1988) and fibre numbers have been correlated with tumour incidences (Churg & Stevens, 1988).

Oberdorster *et al*. (1988) demonstrated translocation of fibres less than 16μm in length and 0.5μm diameter into lung lymph nodes and fibres of less than 9μm and similar diameter into lung lymph in the dog. Lee *et al*. (1981) showed similar translocation of smaller fibres into the lymph nodes of the rat, hamster and guinea pig following exposure to a number of fibre types. From here it is possible for such fibres to be transported to other regions of the body.

Understanding of the mechanisms and ability of different fibre types to translocate and the extent of these pathways in different species is very limited. Evidence suggests that it is frequently the fibres which are translocated away from the site of deposition which are important in the development of disease (Chang *et al.,* 1988).

Dissolution of fibres deposited in the respiratory tract may occur either in lung fluid or within lung cells (e.g. alveolar macrophages) and contributes to long-term clearance. No further consideration of dissolution is given here since this is related to fibre chemistry.

The above clearance pathways will result in fibres entering the gastrointestinal tract and the lymphatic and blood systems, from where they may be transported to other body tissues.

FACTORS MODIFYING CLEARANCE

Particle overload - Animal studies have shown that alveolar mechanical clearance is impaired or even prevented at high burdens of insoluble particles and this is implicated in the development of lung tumours of epithelial cell origin, in the rat specifically. This has been reviewed recently for non-fibrous particles (Hext, 1994). 'Overload' can occur also with fibres (Bolton *et al.,* 1983; Jones *et al.,* 1988) since it is a function of lung burden, particle size/surface area and potential biological reactivity. The consequential effects observed are attributed to the particulate burden and not necessarily to the substance *per se*. Under these conditions, the relevance to man of the development of overload-induced lung tumours in rat experiments is questionable.

Inflammation and other lung disease (e.g. fibrosis, emphysema) will influence clearance rates and mechanisms. For example, associated with on-going inflammation is a reduction in alveolar macrophage mediated mechanical clearance. This may be due to the presence of cellular factors which affect

macrophage mobility. Large species differences exist in alveolar macrophage activity, phagocytosis and in cellular inflammatory responses (Warheit *et al.,* 1988). Which species best simulates the human response has yet to be determined.

PERITONEAL CAVITY

The behaviour of fibres in the peritoneal cavity is a significant research area as an intraperitoneal injection assay is being proposed as a method for testing the potential carcinogenicity of fibrous materials (TGRS, 1994). This arises from the view by some workers that inhalation testing in animals is insufficiently sensitive for assessment for classification purposes of the carcinogenic potential of fibres. However, if a non-physiological route of administration is to be used for testing purposes then the relevance to the normal route must be established and discrepancies highlighted. Hence the increasing interest expressed in the intraperitoneal route of administration.

DISTRIBUTION AND BIOPERSISTENCE OF FIBRES FOLLOWING INTRAPERITONEAL INJECTION

To fulfil the requirements of fibre number in intraperitoneal studies, the masses injected are frequently in excess of 10mg. In more than 80% of rats (n>500) injected with either fibres or dust particles at these high levels, nodules consisting of the injection material were seen in the animals at post-mortem (Collier *et al.,* 1994). These nodules were seen throughout the studies even in the longest lived animals.

Initial studies in rats on the distribution of glass fibre following intraperitoneal injection have shown a rapid uptake of fibres to the surface of the peritoneal organs (Morgan *et al.,* 1993). However, the mass of fibres used (0.84mg) was lower than that normally used for such studies. More recent studies (Collier *et al.,* 1994) at higher doses (10 and 30mg), have shown that up to a dose of 1.5mg there is uptake to the surface of the peritoneal organs, but beyond this level nodules begin to form in proportion to the mass over 1.5mg. The effect of multiple injections (a frequent requirement for intraperitoneal tests) on distribution is not known.

Biopersistence in the peritoneal cavity is currently being compared to that in the lungs. In the lungs of rats, long experimental glass fibres (>20μm) were found to dissolve, whereas short ones dissolved to a lesser extent (Eastes *et al.,* 1995). The explanation for this behaviour may involve uptake of the shorter fibres into alveolar

macrophages. However, when fibres were recovered from the diaphragm following intraperitoneal injection, no length dependent behaviour was found, and all fibres showed dissolution rates similar to short fibres in the lung (Collier *et al.,* 1994). Preliminary results indicate that the material in the nodules (representing the majority of the mass in the peritoneal cavity) shows similar behaviour to fibres on the diaphragm.

The role of the two fibre populations (those in nodules and and those associated with peritoneum) in tumour production is not known. Preliminary results suggest that nodules from animals injected with different fibrous materials show different types of cellular infiltration. The presence of mesothelial cells in these nodules may be a prerequisite for mesothelioma induction but the relation of the fibres and their dimensions to the cellular response in the peritoneal cavity requires further investigation.

An inverse relation between the quantity of non-fibrous material present and the tumorigenic potential of fibres has been demonstrated. The differential distribution of fibrous and non-fibrous dusts in relation to biological responses is not known.

TRANSLOCATION FROM AND WITHIN THE PERITONEAL CAVITY

Preliminary results in rats show little clearance of radioactive fibres from the peritoneal cavity, compared with some evidence for the clearance of powdered fibres (Collier *et al.,* 1994). In studies with crocidolite fibres of different lengths, Moalli *et al*. (1987) showed that, in mice, short fibres (<2μm in length) were able to enter the mesothelial lymphatic plexus *via* stomata in the diaphragm, whereas longer fibres blocked the stomata and caused inflammation and mesothelial cell proliferation. Later studies in mice by Goodglick and Kane (1990) have shown that short fibres are also capable of producing inflammation and mesothelial cell damage if the normal clearance mechanism *via* the stomata is 'overburdened'. Recent studies by Collier and co-workers have indicated that fibre length is a determining factor in intraperitoneal fibre carcinogenesis, with significantly increased mesothelioma incidences for fibre preparations containing a higher fraction of longer fibres. In lifespan studies, translocation of material to the mesenteric lymph nodes was observed for MMMF types and non fibrous dust, but not for crocidolite fibres. The reasons for these differences in behaviour may be related to fibre length and may be significant in the tumorigenic response.

Knowledge is lacking of the distribution and translocation of fibres within the peritoneal cavity of other experimental species.

RESEARCH NEEDS

The majority of work conducted in experimental animals has used the rat and much comparable information is lacking in other experimental species. While this is seen as an area for future experimentation, it is not seen to be a high priority with respect to animal/man comparisons for deposition. Sufficient data exist from experimental studies and mathematical modelling to describe adequately deposition patterns within the various regions of the respiratory tract.

Current knowledge of mechanical clearance is also adequate for many purposes. However, the influence of fibre length on clearance (mechanical and dissolution) and the effects of 'mixed dust' exposure (fibrous and non-fibrous), which can occur in man, suggest that experiments in animals may be required to advance understanding of the relation, if any, between mixed dust exposure and the development of lung disease. The apparent modifying action of non-fibrous dust on tumour development by fibres in the peritoneal cavity adds support to the need for further investigations of this type.

Translocation of fibres has been a consistent finding in experimental studies, and also occurs in man. This is an important process contributing to the development of lung disease in animals and man (and peritoneal effects in rats) but the mechanism(s) by which this occurs is poorly understood. Elucidation of the mechanism(s)/pathway(s) is considered to be a priority. A comparison between the rat and hamster is advocated since this may contribute to understanding of the recently observed apparent difference in sensitivities of these two species to the development of lung tumours (rats) and mesotheliomas (hamsters; Ellouk & Jaurand, 1994).

Intraperitoneal injection has become an important route for assessment of fibre activities and pathological responses for regulatory purposes, as described earlier. Distribution and clearance of different fibre types and sizes following single or multiple injections requires investigation. This will also help understanding of the relation between fibre populations in nodules or on the peritoneum and the induction and progression of biological responses, especially mesothelioma induction.

Preliminary studies with experimental glasses have indicated differences in biopersistence between the peritoneal cavity and lung. These should be

investigated further for other fibre types. If biopersistence is markedly different between the two regions, the applicability of the intraperitoneal studies must be questioned since it may have major implications for the promulgation of intraperitoneal testing of fibres for classification purposes.

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ANIMAL MODELS FOR HUMAN HAZARD ASSESSMENT

G H Pigott and R C Brown

INTRODUCTION

One of the major problems is that the very concepts of risk and hazard were devised for chemical entities and there is a need to examine their relevance to a group of materials whose effect may be primarily determined by their physical form. Although fibres can undergo or catalyse chemical reactions, the bulk of evidence suggests that fibrous habit, the size of the fibres and their durability (biopersistence) are the main determinants of pathogenic activity. Any biopersistent fibre with the appropriate dimensions and delivered in sufficient quantity can produce both fibrosis and tumours of the pleura and lung parenchyma though the surface properties of the fibre may influence incidence rate. The skin irritation caused by fibres may not require a persistent fibre but dimension is again critical. It should be noted that any hazard posed by fibrous materials is a function of a number of inherent properties including their ability to liberate a cloud of respirable fibres; this is determined by the original content of fine fibres, the presence of loose fibrous dust and the friability of the longer fine fibres.

Two forms of cancer are associated with human exposure to some types of asbestos - bronchial carcinoma and malignant mesothelioma. The former shows a very strong association with co-exposure to (asbestos) fibres and cigarette smoke while the latter shows no association with smoking. Human mesothelioma can also be caused by exposure to fibrous erionite. Clearly different processes will be required to identify any hazard relating to these disparate activities.

IRRITATION

The skin effects of fibres are perhaps the simplest to discuss. Asbestos has been known to cause ‘warts’ or corns since the early part of the century; though there is very little literature on this subject the lesions have been reported as

characterised by hyperkeratosis (Alden & Howell, 1944). More familiar is the transient skin irritation experienced when handling many types of synthetic mineral fibres. This irritation is mechanical caused by penetration of the skin; it is of limited medical significance as it does not persist after exposure ceases. Nevertheless it is an important problem for occupational exposure as employees suffer discomfort and may leave the industry.

Assessment of the potential to cause this type of irritation is difficult in animals and only limited attempts have been made to develop a screen. The normal protocols for assessing chemical irritation are not appropriate and exonerate mineral fibres. Since this property is readily, safely, and most appropriately addressed by direct assessment in man the need for animal experiments may be doubted. Experiments with glass fibre (Heisel, 1976) have shown that the potential for irritation is dependant on diameter although it is generally agreed that there is also a materials component, possibly relating to the stiffness of the fibre concerned. Stiff fibres with large diameters are more able to penetrate skin and are thus more irritant than thinner more flexible fibres. With most types of mineral fibre the potential for irritation is largely lost at diameters less than 5μm and in this respect this property runs counter to the other effects (see below). There is no pressing need for additional experimentation to elucidate this particular problem with fibres.

Irritation of the upper respiratory tract and larynx and of the eye has also been recorded in response to synthetic mineral fibres. There is no reason to suppose that the mechanism, and therefore size distribution of importance differs in any respect from that for irritation of the skin.

FIBROSIS

Fibrosis and lung cancer are the more important hazards to control and considerable effort has been put into modelling the behaviour of new materials and any likely response to them. There is no convincing evidence in humans that fibres can express their potential for carcinogenesis or fibrogenesis by other than the inhalation route except in very exceptional cases such as surgical introduction. The greatest efforts have therefore concentrated on responses following inhalation exposure.

Pulmonary fibrosis was the first recognised response to fibrous dusts, though fibrosis can also occur following exposure to various non-fibrous materials. This

effect of fibres was successfully modelled in the 1930's in Gardner's laboratory in several species (published posthumously by Vorwald *et al.,* 1951) and these observations have since been repeated in many laboratories. Even in the early days the logistical problems of inhalation exposure led to attempts to use simpler means and it was established that fibrosis could also be demonstrated when samples were injected into the peritoneum or introduced into the lung by intratracheal injection of suspensions.

There are probably two components to fibrogenesis; the first is materials dependent. Some materials, notably crystalline silica and some forms of asbestos, produce a progressive fibrosis, which may continue long after exposure has ceased and in which large areas of lung tissue may be obliterated. There is also an effect of fibrous habit. Indeed Gardener showed that milling chrysotile asbestos destroyed its fibrogenicity and Wright and Kuschner (1977) showed that, for both glass fibre and asbestos, long fibres are more fibrogenic than short although long asbestos fibres produced a more severe fibrosis than long glass fibres. The mechanism for this is not formally established though it is probable that a combination of incomplete phagocytosis of long fibres leading to potential cytokine release and mechanical damage is responsible for the effects seen. There are several short-term assays which can determine the fibrogenic properties of fibres (McClellan *et al.,* 1992).

CANCER

Assessment of the carcinogenic potential of fibres has received the most attention. Even early inhalation studies repeatedly showed an increase in the incidence of lung tumours in rats (e.g. Gross *et al.,* 1967; Reeves *et al.,* 1971, 1974). Reeves *et al.* also showed that the rat was the most sensitive species and failed to demonstrate significant tumour incidences in rabbits, guinea-pigs, gerbils or mice. In general with inhalation experiments the excess of lung tumours is small and the latent period long, though Davis *et al*. (1986) and Davis and Jones (1988) have shown that exposure by inhalation to long fibre preparations of asbestos produced a considerably higher yield of tumours than did shorter fibres. This is probably the explanation for the negative studies reported with some asbestos samples. These materials had been extensively milled to produce a highly respirable cloud which, however, contained fewer long fibres than many atmospheres encountered in industry. The position with mesothelioma is less clear cut. Wagner *et al*. (1974) were able to demonstrate mesothelioma in rats following inhalation of asbestos fibres even after periods as short as one day.

The largest series of investigations yet undertaken on man-made fibres was commissioned by the man-made fibre industry and carried out at The Research and Consulting Company, Geneva (RCC). While the protocols were based on the OECD recommendations (OECD, 1981) they were considerably extended.

The first of the RCC experiments used refractory ceramic fibres (RCF). To produce suitable material for inhalation the ceramic wool was prepared using two water elutriation stages after carefully grinding bulk fibres. This removed the shot, maximised the proportion of rodent respirable material and selected longer fibres than those previously studied though the samples still contained a proportion of non-fibrous particulates. Later experiments used glass-, rock- and slagwool and the fibres for these experiments were purified using a different water based system. The inhalation methods used were based on those described by Bernstein *et al.* (1980). The results from these studies may be summarised as showing that an exposure to ceramic fibres at 30mg/m^3 (the estimated Maximum Tolerated Dose - MTD) can cause mesothelial tumours in both rats and hamsters and lung tumours in rats. The other fibres produced no significant increase in lung tumours in the rat and have not yet been tested in the hamster. The size and dose characteristics prevent a clear attribution of any differences between different fibres to material considerations, most importantly they do not allow any comparison with asbestos (see Bunn *et al.,* 1993; Glass *et al.,* 1993).

However in one respect these experiments suggest a useful model system for future work. In the initial study RCF1 induced a significant tumour yield whereas RCF4 did not. They were actually samples of the same material but RCF4 had been subjected to prolonged heating to simulate after service fibre, a process which resulted in its detoxification. Thus examination of these fibres may give further information on the importance (or otherwise) of surface chemistry although the initial fragility of RCF4 may preclude a definitive answer in this case.

Experience with these experiments has led to a second series of hamster experiments due to start at the end of 1994; new positive controls and better methods of estimating the MTD are being used.

The expense and problems with inhalation testing encouraged the early development of alternative models. Wagner (1962) demonstrated that, at least for mesothelioma, a moderate to high incidence of tumours could be obtained following intrapleural injection of a bolus dose of fibre followed by a prolonged holding period. In a series of experiments using implantation of fibre samples into the pleural cavity Stanton and Wrench (1972) and Stanton *et al.* (1977) also

induced tumours in moderate to high yield. This response is not limited to the pleural cavity and there has been extensive use of intraperitoneal injection notably by Pott's group in Germany and also by Davis and colleagues in the UK. In all of these models tumour yield is higher than that seen after inhalation exposure though the latent period remains long so that these experiment do not shorten the time needed for evaluation. All of these injection and implantation experiments have shown that activity is dependent on fibres longer than some value of around 10μm and thinner than 0.25μm; activity in any sample can be predicted from the content of these fibres. No universally accepted short-term *in vivo* or *in vitro* predictive screen has so far been developed.

PROBLEMS WITH HAZARD IDENTIFICATION

There remain very serious gaps in knowledge of the application of screens and severe limitations in the screens themselves. The need to identify 'carcinogenic activity' has led to the distinction between the potential to cause bronchial carcinoma and mesothelioma to be blurred or ignored. The mesotheliomas produced in rats are close histological correlates with the human tumour whereas the lung tumours seen in response to fibres are generally adenomas or adenocarcinomas rather than the bronchial carcinoma seen in man. This may reflect different mechanisms in operation between the species although the incidence of human adenocarcinomas may be increasing. The probability that there are different aetiological mechanisms responsible for the lung and mesothelial tumours must also be recognised. Asbestos may act more as a co-carcinogen or promoter in the induction of bronchial carcinoma and parenchymal lung tumours whereas it is probably a complete carcinogen in the induction of mesothelioma. These distinctions are not well understood and a model for the induction of bronchial carcinoma by fibres remains elusive.

There are other significant deficiencies in the models which are available. From the results obtained to date consensus has arisen that the carcinogenic effects of mineral fibres depend on the dose of 'long thin durable' fibres reaching the alveolar region of the lung, though there is no consensus on the exact definition of these terms. Recognition of the importance of fibre size substantially depends on rat studies, both from implantation and inhalation. Extrapolation of the results from the latter route is complicated by the different size-selective properties of the rat and human respiratory tract. Maximum alveolar deposition for both species occurs at 0.5-1μm mineral fibre diameter (with length being much less important as a determinate) but the respirable limit for

mineral fibres in rat is probably close to 2μm whereas for humans it is generally accepted to be about 3μm diameter. Experiments with non-fibrous particulates (in particular with titanium dioxide, long accepted as a 'negative control' material) have shown that the rat lung is particularly susceptible to the phenomenon of 'overload'. In this condition the normal clearance mechanism for particulates is severely impaired and there is a significant and progressive build up of dust deposits in the lung in a manner not seen in normal physiological states. This can cause what is generally accepted to be an artefactual neoplastic response. While the human lung might show a similar response to the very high dust levels causing 'overload tumours' this would not occur at any normal tolerable exposure. The supralinear response of the rat lung to inhalation exposure places a limitation on the dose enhancement which can be used in inhalation studies. As it is a normal feature of toxicology studies that the dose is enhanced to compensate for the relatively small group sizes (and short lifespan) of rodents in carcinogenicity studies, this reduces the sensitivity of the assay. Proponents of injection tests, whether intratracheal or serosal, claim that these studies bypass both the fibre size and sensitivity limitations inherent in inhalation studies.

The reservations relating to the use of serosal tests are well known; they bypass the normal pulmonary defence mechanisms and apply a very large quantity of fibre as a bolus dose. Frequently these studies have expressed the dose on a mass basis and the inocula therefore contained variable numbers of fibres. If a constant number of fibres is used then the mass injected may vary by up to three orders of magnitude, depending on the size characteristics of the fibres involved. These large mass doses, which are necessary only for relatively coarse diameter fibres could induce tumours by a non-specific mechanism related to local irritation, such as that recognised in other areas of toxicology and described by Grasso and Golberg (1966).

The use of serosal tests therefore introduces a tautology. It is claimed that the production of tumours after intraperitoneal or intrapleural injection demonstrates the biological activity of the inoculated fibres. These must therefore represent a risk and have been correctly identified as carcinogenic. The absence of identified fibre samples universally recognised as negative for carcinogenicity precludes a proper evaluation of this system and it is an equally tenable hypothesis that these tests do not show sensitivity to a wide range of carcinogenic fibres but merely lack specificity.

RESEARCH NEEDS

Interpretative problems with animal tests used as predictive screens for toxic properties of fibres are substantially exacerbated by the lack of any knowledge of the mechanism by which toxicity, particularly carcinogenicity, occurs. Thus all test systems are empirical. Even the information that does exist to assist a study of mechanisms is currently relatively crude. Most investigators concur with the hypothesis derived by Stanton *et al.* (1977) and Pott (1978) that the dimension of fibres is important in determining carcinogenicity, or more specifically mesothelioma (the difficulty of extending this to other forms of lung cancer is often overlooked due to the lack of a sensitive and specific model for the human type of tumour). At best these studies have shown an association between the presence of particular size fractions within the test sample and the subsequent appearance of tumours.

The first step in the development of a credible animal model would be to refine further the size dimensions believed to be important, possibly by the use of an *in vitro* system, and then synthesise samples of fibre with very limited spread of dimension which would be predicted to be active and inactive *in vivo.* The various available screens could then be used to assess the validity of these observations which would then need confirmation from reference to what is known of the fibre counts in the epidemiological studies conducted, as a validation of specific tests (as predictive screens).

The problem with such an approach is that it is effectively self-fulfilling; it tends to support a given hypothesis by selecting a suitable model. An ideal animal screen would act as a surrogate for human response. It therefore needs the same size selective characteristics as the human respiratory tract, should show the same dissolution capability as the human lung for deposited materials, clearance should be at the same rate and routes as that in the human and it should show the same spectrum of disease responses. It must be capable of significant dose enhancement and should considerably shorten the times to produce disease in order to prove useful as a screen. The prospect of developing such a system is negligible.

Pragmatically therefore there are three approaches which, though falling short of the ideal, should give increasing assurance that the hazardous properties of any novel fibrous material could be evaluated.

In the first place, preparation and provision of a number of fibres accepted as negative control materials would be a valuable aid in the selection of an animal

system as a surrogate for human exposure. Careful experiments could allow the definition of a protocol which could then be used, probably including a limit dose beyond which non-specific responses might reasonably be expected. The selection of protocols for both injection and inhalation test systems would be desirable so that some hierarchy of testing could be constructed.

Secondly, the information relating to fibre size could be further refined and specially prepared sized samples could be used in the selected animal screen(s). This would allow optimisation of the selected screen to detect activity in a new material. Subsequent experience may then render the screen redundant when sufficient correlation between material science and biological activity is available for accurate prediction.

Thirdly, the information derived from the above may be used to elucidate the mechanism by which fibres induce tumours. This would probably commence with a series of experiments similar to those described by Brand *et al.* (1976) for other solid state carcinogens. These should address such questions as :

> How long do fibres need to be present before an irreversible change occurs?
>
> How many fibres are necessary to induce the change?
>
> Do these need to be the same fibres during the critical period or will serial exposure to even a soluble fibre pose a risk?
>
> What, if any, species differences are there in responses?

The above represents a substantial programme which realistically would take a considerable time. Maximum benefits in terms of risk assessment could be obtained if in the short-term the programme were to concentrate on identification of fibre sizes of importance in the induction of any pathology and the dependence (if any) on the model species used. This would allow the economical use of animals for further investigation of mechanisms and potentially render the need for an animal based screen for new materials redundant.

Development of a model system for the induction of asbestos-induced bronchial carcinoma could also resolve the question of whether this tumour arises from a totally different mechanism than that for mesothelioma and also determine whether it is true that the control of fibrosis will also control this tumour.

One underlying assumption in the above is that the durability of the fibres concerned is important. There is a therefore a significant need to develop systems

to establish the rate at which fibres can be dissolved/mechanically disrupted/fragmented *in vivo*. This is a potentially most difficult problem. If the durability of fibres is of importance in disease induction then it is necessary to establish whether this value is absolute or (as seems more probable) relates to a proportion of the lifespan of the target species; the difference this would make to extrapolation from animals to humans is obvious.

The investigations outlined above go some way to answering research needs though to provide a satisfactory answer to these questions it may be necessary to repeat studies in longer lived species than those normally employed. These experiments would be difficult, time consuming and therefore expensive.

Finally the overall determination of durability, which to produce an accelerated model of dissolution has usually been studied in *in vitro* systems using surrogate media and elevated temperatures, will need to be compared and hence validated in several species. Again standard protocols for the *in vitro* and *in vivo* determination of fibre durability are under development.

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HUMAN EVIDENCE, DOSE RESPONSE AND MECHANISMS

A R Gibbs

INTRODUCTION

The main reactions of the human lung and pleura to inhalation of mineral fibres considered herein are lung fibrosis, lung cancer, pleural plaques, diffuse pleural fibrosis and malignant mesothelioma. There are different mechanisms and dose-responses for each of these diseases. In general pleural reactions occur at much lower doses than those in the lung parenchyma.

It appears that different types of fibre cause a wide range of biological responses which are related to factors such as physical dimension, durability, solubility, tensile strength, surface charge, mineral composition and mineral structure. Fibres to be considered in this paper include natural fibres such as asbestos, zeolite, wollastonite, palygorskite and sepiolite, and the group of man-made mineral fibres (MMMF).

INORGANIC NATURAL FIBRES

ASBESTOS

This family of minerals is the best studied, but there are many gaps still present in knowledge concerning dose-responses and mechanisms. Since they have different potentials for causing human disease it is important to distinguish the two major groups of asbestos:

Serpentine - the major constituent is chrysotile

Amphibole - this group includes amosite, crocidolite, tremolite-actinolite and anthophyllite.

Industrial processes may alter fibre dimensions and other properties; therefore, when trying to assess health risks from a particular fibre, epidemiological evidence from the mining or production of a fibre with its associated minerals and other contaminants, and from end users, should all be considered. One particular example is the contamination of some Canadian chrysotile by tremolite and the role of the latter in causing mesothelioma amongst miners and millers (McDonald *et al.,* 1993). It seems that the processing of the chrysotile ore may remove variable amounts of the tremolite so that different chrysotile products may contain different proportions of tremolite. Therefore, workers exposed to such products will have different risks of developing asbestos related disease. Further pathological and mineralogical studies are required in the various 'chrysotile' exposure situations to clarify the role of tremolite *versus* chrysotile in causing lung and pleural disease.

Another difficulty in the epidemiological study of exposures to a particular mineral fibre is that exposures are frequently mixed; this is not always appreciated and there is usually little accurate information concerning airborne fibre levels. Fibre burden studies of lung tissue can be extremely useful in verifying the type and quantity of exposure because amphibole fibres are retained in the lung tissues for many decades. Initially the Rochdale textile plant in the UK was considered to be a 'chrysotile' plant but electron microscopic analysis of lung tissues from workers at this plant showed substantial quantities of crocidolite (Wagner *et al.,* 1982). It became apparent later that significant amounts of crocidolite had been used in the plant; chrysotile is relatively rapidly cleared from lung tissues and fibre burden studies therefore do not give an idea of the level of exposure. However, if the chrysotile is contaminated with tremolite, which is preferentially retained in the lung, then fibre burden studies which show the tremolite can be used to provide an index of chrysotile exposure. Fibre burden studies provide the only measure of retained mineral fibres in the lung and studies have shown correlations with disease patterns.

Lung Cancer

Although some animal studies have suggested that chrysotile and amphiboles are equally pathogenic on a mass but not number basis, this does not appear to be the case in humans. Indeed some scientists regard chrysotile as relatively innocuous. The differential clearance between amphiboles and chrysotile from the lung is probably the reason why the latter is much less pathogenic than the former. Chrysotile appears to be cleared from the lung in a matter of months after

cessation of exposure and it appears to reach a plateau even with continuous exposure, whereas amphiboles tend to be retained and accumulate with increasing exposure.

For most industries which used predominantly chrysotile, the slopes of the exposure response curves for lung cancer are shallow with no excess risk found below 50 f/ml years in the Quebec mining and milling industry and none at all in the UK and Zimbabwe cement plants and UK friction products plant. High levels of exposure to chrysotile uncontaminated with amphiboles have occurred in the Transvaal, Swaziland and Zimbabwe and there have been very few associated lung cancers (Gibbs, G.W. *et al.,* 1994). There is one situation, however, which stands out from the rest and that is the South Carolina textile plant where the SMR for lung cancer was 199 (McDonald *et al.,* 1983), which is above that for the Quebec miners and millers. This increased risk is not accounted for by tremolite contamination since the Quebec group has a higher exposure to tremolite contaminated chrysotile than the South Carolina group, which is reflected in the higher levels of tremolite in the lung tissues of the former. Other factors might be involved such as contamination by mineral oils and fibre size distributions. There have been increased risks of lung cancer in other industries where mineral oil exposures have occurred. There is no known evidence indicating that the association of mineral oils alters the size distribution or durability of chrysotile. Industrial exposures to chrysotile tend to be to long thin fibres but even so, in contrast to amphibole fibres, clearance occurs relatively quickly (Churg & DePaoli, 1988). The papers from a recent workshop on the health risks from exposure to chrysotile asbestos which have recently been published explore some of these issues (Gibbs, G.W. *et al.,* 1994).

Exposures to amphiboles in various industries have been associated with an increase in lung cancer risk which is generally much higher than for similar industries using chrysotile (Hughes, 1991) and there is limited information which suggests that crocidolite has a greater effect than amosite (Sluis-Cremer *et al.,* 1992).

There is still some controversy as to whether asbestosis is a necessary precursor of asbestos related lung cancer. Some scientists argue that any exposure to asbestos will increase the risk of lung cancer regardless of whether fibrosis (asbestosis) is present or not; others argue that the risk only increases when the exposure is enough to produce asbestosis even though asbestosis might not be present, and yet others believe that the risk only increases when asbestosis is actually present. Although this question has not been completely answered, the majority of the

evidence so far indicates that the latter postulate is the correct one and this indicates that there is a threshold of exposure to asbestos which can be tolerated before the risk of lung cancer increases. The subject has been reviewed recently by Churg (1993). When asbestosis is present, the risk from asbestos exposure and cigarette smoking appears to be more than additive.

It is interesting that Churg and Stevens (1993) could not find amosite fibres in the airway mucosa of heavily exposed non-smoking shipyard workers and insulators but they did find a variety of nonasbestos particles. This suggests that asbestos does not cause lung cancer by a direct effect on the airway epithelium. The majority of the studies looking for chromosomal aberrations in human bronchial cells following chrysotile exposure have been negative. Further studies of this type are required for other cohorts.

Lung fibrosis

The role of chrysotile in causing lung fibrosis is questionable. Fibrosis does occur with high exposures, for example in the Quebec chrysotile industry, but the level of fibrosis correlates much better with tremolite than chrysotile fibre burden. Other studies of asbestosis have shown correlations between amphibole but not chrysotile fibre burden. Epidemiological studies of plants where chrysotile has been used almost exclusively, show no excess of non-malignant asbestos related disease. There is no information comparing the fibrotic responses to amosite with those to crocidolite.

In animals there is evidence to show that long fibres are more pathogenic than short ones in producing lung fibrosis. This has not been confirmed in the small number of human studies conducted (Churg *et al.,* 1989).

Pleural Plaques

Pleural plaques (discrete, dense fibrous areas on the pleura) are associated with relatively low exposures to asbestos, and in some parts of the world these can be environmental. Pleural plaques are observed in chrysotile exposed populations but it is not certain whether they are caused by the chrysotile *per se* or trace amounts of amphibole. In Quebec,where the chrysotile asbestos mines are located, it has been shown that the ore contains very small amounts of tremolite and that this is higher in some of the mines around Thetford than

those around Asbestos. Interestingly, the rate of pleural calcification is higher in the Thetford than in the Asbestos mine workers. Case studies of subjects found to have pleural plaques at post mortem, but not dying of an asbestos related disease, have shown normal levels of asbestos in approximately 50% of the lung tissues. It has been suggested that the longer and heavier the exposure the more extensive the plaques will be. (However, no correlation has been found between the extent of pleural plaques and fibre levels within the lung tissues in a small series of cases.) A study of fibre burdens in the lungs of Quebec chrysotile miners and millers has suggested that exposure to high aspect ratio* tremolite fibres is important in their development (Churg *et al.,* 1993).

The relation between the development of pleural plaques and the likelihood of developing malignant disease of the pleura or lung is disputed. On balance there appears to be no direct relation and their presence does not indicate more than the fact that there has been asbestos exposure. There are certainly populations where there are high incidences of pleural plaques without mesotheliomas; for example, the individuals located around the Finnish anthophyllite mine in Paakila. The recent study by Tuomi *et al.* (1989) which found that anthophyllite was the most frequent type of asbestos found in the lungs of six of 19 Finnish mesothelioma cases can be criticised in so far as their method underestimated fibres less than 0.3μm in width, which would have included chrysotile and some crocidolite fibres.

There are other causes of pleural plaques such as exposures to talc (uncontaminated by amphiboles) and mica; there are also idiopathic cases. In general those not related to asbestos have not been well studied.

Diffuse pleural fibrosis

Studies of asbestos related diffuse pleural fibrosis are limited. In general they indicate that it can follow fairly low exposures to amphibole rather than chrysotile fibres (Stephens *et al.,* 1987). It is not understood why some subjects develop diffuse pleural fibrosis rather than localised plaques. There is some evidence to suggest that in populations where diffuse pleural fibrosis occurs mesothelioma may develop, whereas in those where there are only pleural plaques mesothelioma is unlikely to occur. Further studies are needed in this area.

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* Aspect ratio is the length to width ratio of a fibre.

Mesothelioma

It has been estimated that about 80 to 90% of mesotheliomas in the UK have been caused by asbestos exposure and that these can nearly all be accounted for by exposures to amphiboles (Gibbs, 1991). The study of the gas mask workers in the UK is being updated. This cohort of workers was exposed to crocidolite asbestos during the manufacture of gas masks and has shown very high rates of mesothelioma. In contrast, no definite mesothelioma has been encountered in those workers who only assembled gas masks containing chrysotile filters.

The continued increase in incidence of mesothelioma in many parts of the world is of concern and suggests that the cumulative exposure necessary to cause the disease is much lower than previously thought. Some cases of mesothelioma in the UK have been linked to neighbourhood exposures to amphibole asbestos. The Leeds Mesothelioma Study is a case control study which includes approximately 150 to 200 cases and a similar number of controls from three districts in Leeds. The population of two of the districts includes subjects who have either worked at or lived near the Roberts Asbestos Factory at Armley. In addition to detailed exposure histories, pathological examination and fibre burden studies of the lung are being performed. This will provide useful data on neighbourhood and paraoccupational cases of mesothelioma as well as those associated with direct exposure.

Recently in the UK there has been an increase in mesotheliomas in younger subjects which requires further study of the exposure histories, pathology and fibre burdens. (Peto *et al.,* 1995)

The gradient of mesothelioma risk for fibre type appears to be in the following order:

erionite (a non asbestos fibre) > crocidolite > amosite/tremolite > chrysotile

In the relatively small number of mesotheliomas which have occurred in the Quebec miners and millers, fibre burden analyses have shown very high chrysotile and tremolite concentrations, of the order seen in asbestosis. Churg and Wright (1989) compared the mineral burdens of nine shipyard and insulation workers with mesothelioma to nine cases of exposure-period-matched chrysotile industry workers with mesothelioma and to nine pairs of workers with asbestosis. They found that the chrysotile workers with mesothelioma had a median chrysotile/tremolite level of 290 million fibres/g dry lung, which was 400 times the median crocidolite/amosite burden of the shipyard and insulation workers with

mesothelioma (0.7 million fibres/g dry lung). The median chrysotile/tremolite fibre level in the chrysotile industry workers with asbestosis was 110 fibres/g dry lung as compared with a median crocidolite/amosite level of 26 million fibres/g dry lung in the shipyard and insulation workers with asbestosis. They concluded that with crocidolite/amosite exposure mesothelioma occurred at a much lower fibre burden than asbestosis, whereas with chrysotile/tremolite exposure mesothelioma and asbestosis occurred at similar fibre burdens. This would suggest that in present day working conditions exposure to chrysotile ore products is unlikely to result in mesothelioma.

A study of lung burdens of subjects predominantly exposed to amosite at the Cape Boards plant at Uxbridge showed mean amosite fibre counts (in millions of fibres/g dry lung) of 358 in exposed workers without disease, 1035 in workers with mesothelioma, and 1483 in workers with lung cancer. Cases of moderate to severe asbestosis showed amosite counts above 500 million (Gibbs, A.R. *et al.,* 1994).

Animal studies have shown that the optimal dimensions for the induction of mesothelioma are a length of > 8μm and a diameter of < 0.25μm (Davis, 1989), but the few human studies performed have given conflicting results. McDonald *et al.,* (1989) concluded that fibres > 8μm in length were associated with mesothelioma but that short fibres played no role. On the other hand the studies of Churg did not find that mesothelioma was associated with high aspect ratio fibres. This area is worthy of further study.

NON-ASBESTOS RELATED MESOTHELIOMAS

There is evidence for a background incidence of mesothelioma unrelated to asbestos and there are certainly case of mesothelioma where the occupational histories have been negative for asbestos exposure and lung fibre burden analyses have been normal (Gibbs *et al.,* 1989). The causes of mesotheliomas unrelated to asbestos exposure are unknown at present. Some rare cases of non-asbestos related mesothelioma have followed irradiation, usually for tumours such as lymphomas and Wilms' tumour, many years before. Other proposed causes have been exposures to inorganic minerals such as silica, beryllium and nickel, organic chemicals, viruses and chronic inflammation. These are for the most part speculative but the role of viruses should be considered further. A recent study of human pleural mesotheliomas identified Simian Virus 40-like DNA sequences in 60% of 48 mesotheliomas studied (Carbone *et al.,* 1994). The authors suggested that the virus might act independently or as a co-carcinogen with asbestos.

In the Cappadocian region of Turkey there has been a high incidence of mesothelioma from environmental exposures to erionite and in North America there have been some data that have suggested that erionite deposits have contributed to mesothelioma incidence.

There have been reports also of mesothelioma occurring in sugar cane workers from India and southern Louisiana. It has been postulated that the cause was inhalation of long thin respirable silica fibres released after burning off the cane (Newman, 1983). It would be interesting to perform mineral analyses on lung tissues from some of these workers.

ZEOLITES

Erionite, a fibrous form of zeolite, has produced mesotheliomas, lung cancers and fibrosis in animals after inhalation. Rates of lung cancer and mesothelioma are very high in three villages in Cappadocia where the fibre occurs naturally in rocks and dust. Erionite has a similar size distribution to crocidolite but appears to be more pathogenic (Wagner *et al.,* 1985) which suggests that chemical surface properties are also important.

WOLLASTONITE

Wollastonite is a natural acicular (needle shaped) and fibrous calcium silicate which has been used as a substitute for asbestos. There have been too few studies to draw conclusions about carcinogenic risk. Pleural plaques and lung fibrosis have been described in association with exposure to wollastonite in the ceramic industry.

PALYGORSKITE /ATTAPULGITE

Studies of exposure to these minerals have been very limited but there has been a suggestion of an increased risk of lung cancer (Waxweiler *et al.,* 1988). The physical dimensions of the fibres vary considerably with the source (Wagner *et al.,* 1987); many are short fibred but the palygorskite from Leicester, UK, has almost 20% fibres >6μm in length. Palygorskite derived from different sources shows a range of *in vitro* activity (Nolan *et al.,* 1991).

SEPIOLITE

Sepiolite has a similar structure to palygorskite. It has been used most frequently as litter for domestic pets. The only study published is a cross-sectional one of Spanish sepiolite workers (McConnochie *et al.,* 1993). In this study no radiological evidence of fibrosis or pleural disease was found. No pathological study of these workers has been published.

INORGANIC MAN-MADE MINERAL FIBRES

These include glass-, rock- and slagwools, continuous filament, refractory (including ceramic) fibres, and special purpose fibres. Many of these fibres contain a proportion that is respirable and therefore potentially pathogenic. The number of potentially exposed workers is high and the general public may also be exposed to some of these fibres (Liddell & Miller, 1991).

There have been two large epidemiological studies in Europe and the USA encompassing several plants manufacturing rock-, slag- and glasswools and continuous filament. These studies have indicated a 30 to 40% increase in respiratory cancer in rock- or slagwool workers 20 years or more after first exposure (Enterline *et al.,* 1987; Simonato *et al.,* 1987). There is evidence to suggest that the excess of cancer in rock/slagwool workers is mainly due to the use of slag. However, there may be confounding factors present in some of these plants such as arsenic, polycyclic aromatic hydrocarbons, bitumen and asbestos. There has not been a significant increase in lung cancer risk in the European slagwool workers from the late technological phase where fibre levels were much lower. Both of these studies are ongoing and there will be further updates.

There has been one published study of mineral fibre analysis in workers exposed to MMMF (McDonald *et al.,* 1990). The subjects were from the American MMMF worker cohort referred to above. The study comprised 145 subjects and 112 matched referents; 26% of the workers were found to have MMMF in the lung tissues at relatively low concentrations. There were not enough cases of lung cancer to draw any conclusions with respect to fibre number and neoplasia. There are no other published data on fibre number and size distribution in human lungs from these types of exposure.*

*In an ongoing study by the author lung tissues from a small number of glass fibre workers are being examined.

Mesotheliomas have not so far been associated with exposure to MMMF.

Animal inhalation studies with MMMF have not induced a significant increase in lung tumours and mesotheliomas (IARC, 1988); this may be related to their short durability in lung tissues. Refractory ceramic fibres, which are durable in lung tissues, are an exception; they have produced both lung tumours and mesotheliomas after inhalation in animals (Hesterberg *et al.,* 1991). There appears to be no published study on the effects of refractory ceramic fibres in humans.

RESEARCH NEEDS

There is still debate as to whether or not asbestosis needs to be present before lung cancer risk increases following asbestos exposures. Epidemiological studies of lung cancer cases in combination with pathological and mineralogical analysis of lung tissues should be conducted to determine whether the risk of lung cancer only increases with the presence of asbestosis and if so at what level of fibrosis.

Are there exposures to chrysotile uncontaminated by amphibole fibres such as tremolite? The best way to answer this question would be to carry out pathological and mineralogical studies of lung tissues from workers exposed to 'chrysotile' from a range of locations and industrial processes. This should include miners and millers, textile workers, cement workers, friction product workers and end users. The human lung is an efficient means of filtering out trace amounts of amphibole fibres from chrysotile materials.

The incidence of mesothelioma appears to be increasing and this includes younger subjects. Is this increase genuine or is it accounted for partly or completely by greater awareness, previous underdiagnosis and more recent overdiagnosis? What is the role of asbestos in this increase and does it apply to younger subjects? Studies of UK mesothelioma cases, particularly with respect to the younger subjects, to verify the accuracy of pathological diagnosis, and studies on fibre burden would help answer these questions.

Information is required on the durability of various fibres in human lung tissue since there is good evidence that durability in tissues is closely associated with disease-causing potential. Pathological and mineralogical studies of lung tissues from workers exposed to man-made mineral fibres, particularly slagwools, and end users would provide useful information and might help to predict hazard. Analysis of bronchoalveolar lavage samples might also be utilised.

Recently there has been discussion about the role of Simian Virus 40 in the pathogenesis of mesothelioma. A study of occupational and non occupational mesotheliomas with full non fibrous and fibrous mineral analysis and examination of lung tissues for Simian Virus 40-like sequences would clarify its role.

The presence of pleural plaques in subjects is sometimes used to ascribe certain pleural and lung parenchymal diseases to asbestos exposure, probably inaccurately. Pleural plaque studies to examine the relationship between extent of plaques, lung parenchymal changes and fibrous and nonfibrous particulate burden would clarify this.

In contrast to animal studies there is still no clear cut evidence concerning which fibre dimensions are important in human fibre associated diseases. There is need for further work to evaluate the role of fibre size and dimension in human asbestos related diseases.

Pathological and mineralogical studies of lung tissues from workers exposed to wollastonite, palygorskite and sepiolite are needed to assess their durability in human lung tissues. An associated epidemiological study would also be useful to compare occupational measures of exposure with lung mineral burdens.

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